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ORIGINAL ARTICLES

NITROGEN FIXATION IN RICE SOILS

BY

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(Received for publication on 14th November 1938)

(With one text-figure)

Introduction

In ordinary farming practice rice is grown in the Konkan without the addition of manure or fertiliser to the soil. Sahasrabuddhe and Abhyankar [1936] have shown that cultivated rice soils at Karjat contain more nitrogen than the uncropped soils during the growing period of rice crop. Sahasrabuddhe [1936] later confirmed these findings by laboratory experiments and was further able to show that rice soils carry an inoculum which has the power of fixing nitrogen and that this fixation of nitrogen is helped by the presence of growing roots of rice plants. He did not, however, attempt an elucidation of the nature of this inoculum, which, as has been shown in the following pages, consists of free-living nitrogen-fixers.

Starkey [1929] cites several workers who have shown that tobacco, corn, mustard and some crucifers are benefited by the presence of *Azotobacter* in the soil. He [1931] has also reviewed the evidence indicating that the bacterial life is greatly enhanced in cultivated soils and is virtually at a standstill in fallow. Lipman and Brown [1907], however, failed to show any definite increase in the nitrogen-content of the soil which had been inoculated with *Azotobacter vinelandii* and *A. beijerincki* and cropped with oats, corn and rye in succession. On the other hand, Stranak [1910] showed that potatoes, grain and beets grew excellently in soil inoculated with *Azotobacter*. Although these results are conflicting, yet it seems possible that under suitable conditions of growth *Azotobacter* can fix appreciable amounts of nitrogen.

Sen [1929] suggested the possibility that nitrogen-fixing organisms may live symbiotically in the roots of rice plants, whilst Viswa Nath [1932] is of the opinion that the rice plant itself has the power of fixing atmospheric nitrogen. Recently De [1936], working with a mixed culture of algae and bacteria, came to the conclusion that the fixation of nitrogen in rice soils under waterlogged conditions is an algal process, although he was unable to determine whether the fixation was brought about by the algae themselves or by algae working in symbiosis with bacteria. On the other hand, Bristol and Page [1923] have shown that pure cultures of algae do not possess the power of fixing elementary nitrogen, though Allison and Hoover [1935] have recently claimed this ability for certain *Nostoc*.

The conflicting evidence on the nitrogen recuperation of cultivated soils led us to the re-examination of the problem by direct experiments with pure cultures of the organisms isolated from rice soils at Karjat.

Experimental methods and results

Rice plants with soil about their roots were collected during the rainy season in July 1936, from different plots on the Rice Breeding Station at Karjat ; the soil was submerged at the time when the plants were removed. Isolations from soil samples were made on Ashby's agar using dilution method. The plates were incubated at room temperature, varying from 25° to 27°C. Colonies of bacteria began to appear in the plates after 48 hours, and a majority of these colonies turned dark with age. These dark colonies were further purified and the organism was then grown on slants of Ashby's agar.

For the purpose of inoculation a two days' growth of the organism on Ashby's agar was invariably used. Inoculation was done by scraping growth from agar slants with a sterile needle and transferring it to flasks containing 50 c.c. of Ashby's solution.

Experiment I.—The organism was isolated from samples of submerged soil collected in the immediate proximity of the roots of growing rice plants at Karjat. The transfer of the organism was made into culture flasks each containing 50 c.c. of Ashby's solution, in some of which rice plants were also grown. The experiment lasted for 30 days after which total nitrogen was determined by Ranker's [1927] modification of the salicylic-thiosulphate method. Two series of tests were made in duplicate at different times, and the results are summarised in Table I.

The results show that all the four cultures of the organism isolated from soil samples from Karjat can fix appreciable amounts of nitrogen and that isolate III (Is. III) is an efficient nitrogen-fixer. These results further bring out the interesting fact already reported by Sahasrabuddhe [1936] that growing roots of rice plants markedly enhance the nitrogen-fixing capacity of rice soils.

TABLE I

Amounts of nitrogen fixed by organism isolated from rice soil

Ashby's solution containing	Total nitrogen in mg.			
	Series A		Series B	
	Individual flask	Average	Individual flask	Average
Isolate 0	1·24 1·22	1·23	.. ..	..
Isolate I	1·37 1·41	1·39	0·9 0·9	0·9
Isolate II	1·42 1·48	1·45	0·965 0·965	0·965
Isolate III	1·64 1·62	1·63	0·965 0·965	0·965
Isolate 0+9 rice plants	2·982 3·038	3·01	.. ..	..
Isolate I+9 rice plants	3·097 3·183	3·14	2·576 2·700	2·638
Isolate II+9 rice plants	3·09 3·07	3·08	2·856 2·752	2·804
Isolate III+9 rice plants	3·712 3·648	3·68	3·221 3·285	3·253
9 rice plants alone	1·432 1·408	1·42	1·538 1·522	1·53

In view of the fact that the nitrogen-fixing power of isolate III is quite high, it has been used as inoculum in the experiments that follow.

Experiment II.—There is a definite indication from Experiment I that the association of rice plants with the organism enhances its normal activity to fix nitrogen. It is not known, however, whether the introduction of sterilised soil into the culture flasks exerts a similar beneficial effect on the organism. An experiment was therefore made to study the effect of the association of rice plants and soil on the activity of the organism to fix nitrogen.

Seeds of Kolamba variety of rice were obtained from Karjat and disinfected in 1 in 500 solution of mercuric bichloride for 15 minutes. The seeds were then washed in sterile water and transferred aseptically to sterilised glass chambers lined inside with sterile, damp filter paper. After germination of seed the seedlings were allowed to grow in the glass chambers kept at 32°C. for eight days before removing them to culture flasks.

Five sets of culture flasks each containing 50 c.c. of Ashby's solution were prepared and treated in the following manner :—

(1) Inoculated with Is. III

(2) One gram of sterilised rice soil was added to each of the culture flasks in this set, of which half the number was inoculated with Is. III

(3) Nine rice plants were grown in each of the culture flasks in this set, of which half the number was inoculated with Is. III

(4) In one group containing half the number of culture flasks included in this set, nine rice plants were grown in each culture flask to which one gram of sterilised rice soil was added ; the second group was similarly treated but was inoculated with Is. III and

(5) Same as in the second group of (4) above except that in half the number of culture flasks in this set roots of rice plants were introduced into Ashby's solution for ten minutes and in the other half rice plants were grown for ten days, before inoculating the culture flasks with Is. III.

At the conclusion of the experiment after 30 days the total nitrogen was determined from duplicate samples of each culture flask including the rice plants. The results given in Table II are of two series done at different times.

The results presented clearly show that the organism, in association with rice plants or rice soil or both, is able to fix larger amounts of nitrogen than it does in the absence of such association. Rice soil which has been sterilised does not possess the power of nitrogen fixation since the small difference in its nitrogen content (0·863—0·320 mg.) and that of Ashby's solution (0·320 mg.) may be due to the amount of nitrogen already present in the soil. However, when the culture flask containing sterilised soil is inoculated with the organism, 2·838 mg. (3·733—0·895) nitrogen is fixed. It is not clear how soil can stimulate the organism to greater activity unless it is assumed that this beneficial effect is due to certain minerals and salts such as phosphates supplied by the soil [Waksman, 1931]. Likewise, rice plants themselves are incapable of fixing atmospheric nitrogen, but in their presence the ability of the organism to fix nitrogen is greatly enhanced due probably to the excretion of growth-promoting substances from the roots. Further, in the presence of both soil and roots of rice plants as simulating conditions met with in the field, the organism is aroused to still greater activity as it can fix 5·611 mg. (8·200—2·589) or 6·610 mg. (8·827—2·217) nitrogen per 0·5 gram of mannite utilised in 50 c.c. of Ashby's solution. Another interesting fact

emerging from these experiments is that, when rice plants are allowed to grow in Ashby's solution before inoculation for a period longer than ten minutes, the growth of the organism is adversely affected with the result that the efficiency of nitrogen fixation decreases, *i.e.*, 2·776 mg. (5·365—2·589) nitrogen is fixed when roots of rice plants are introduced for ten minutes in Ashby's solution as against 1·087 mg. (3·676—2·589) nitrogen fixed when plants are grown for ten days.

TABLE II

Effect of association of rice plants and soil on the nitrogen-fixing power of the organism isolated from rice soil

No.	Ashby's solution containing	Total nitrogen in mg.			
		Series A		Series B	
		Individual flask	Average	Individual flask	Average
1	Is. III	1·688	1·699	1·416	1·410
		1·710		1·404	
2	1 grm. sterilised soil	0·863	0·863	0·895	0·895
		0·863		0·895	
	1 grm. sterilised soil+Is. III	..	..	3·712	3·733
		..		3·754	
3	9 rice plants	1·531	1·543	1·680	1·688
		1·555		1·696	
	9 rice plants+Is. III	3·321	3·339	3·415	3·382
		3·357		3·349	
4	1 grm. sterilised soil+9 rice plants.	2·602	2·589	2·230	2·217
		2·576		2·204	
	1 grm. sterilised soil+9 rice plants+Is. III.	8·245	8·200	8·800	8·827
		8·155		8·854	
5	1 grm. sterilised soil+9 rice plants (10 minutes)+Is. III.	5·255	5·365	..	..
		5·475		..	
	1 grm. sterilised soil+9 rice plants (10 days)+Is. III.	3·662	3·676	..	..
		3·690		..	

N.B.—Ashby's solution in these experiments contained 0·320 mg. nitrogen. Nine rice plants in these experiments contained about 1·2 mg. nitrogen.

Experiment III.—The previous experiment has shown that the organism in association with living rice roots is able to fix larger amounts of nitrogen. This experiment was therefore undertaken to ascertain whether any growth-promoting substance is contained in the living roots of rice plants or whether the stimulating effect is caused by increase in available energy due to the introduction of plant residues such as roots. For this purpose two groups of culture flasks, in duplicate, each containing 50 c.c. of Ashby's solution and

a gram of sterilised rice soil were set up ; five c.c. of the extract prepared from the roots of nine of the rice seedlings grown under sterile conditions in Knop's solution was added to one group of flasks and choppings of roots of the same number of plants grown under identical conditions to another group of flasks. After twenty days the total nitrogen was determined from each culture flask, and the results are recorded in Table III.

TABLE III

Effect of rice root extract and choppings on the nitrogen fixing power of the organism

No.	* Ashby's solution + soil containing	Total nitrogen in mg.	
		Individual flask	Average
1	Root extract	1·910	1·809
		1·709	
	Root extract + Is. III	2·376	2·343
		2·310	
2	Root choppings	1·718	1·709
		1·696	
	Root choppings + Is. III	3·085	3·085
		3·086	
**3	9 rice plants growing	2·096	2·095
		2·095	
	9 rice plants growing + Is. III	6·987	6·495
		6·004	
**4	Is. III	2·549	2·499
		2·439	

* Ashby's solution + soil contained 0·874 mg. nitrogen.
** Controls.

In considering the results in the above table, the following points seem to be established :—

(1) The organism is stimulated to greater activity when grown in culture in association with living roots of rice plants as it can fix 3·996 mg. (6·495—2·499) nitrogen as against 1·625 mg. (2·499—0·874) nitrogen fixed by the organism without association. The amount of nitrogen thus fixed is more than when an extract of the roots or their choppings are added to the culture, thus indicating that the stimulation of the organism is brought about in some manner by the living roots and is not due to any substances present in the dead roots.

(2) The addition of the root extract to the culture flasks does not exert any stimulatory effect on the power of the organism to fix nitrogen since the amount of nitrogen thus fixed is almost the same as in the control ; but some

slight beneficial effect is discernible when root choppings instead of the root extract are introduced into the culture flasks. This small increase in nitrogen fixation may be due to the organic matter serving as an additional source of energy.

Experiment IV.—In an earlier experiment indication was obtained that the time for which the living roots of rice plants are growing in Ashby's solution previous to inoculation is an important factor affecting the power of the organism to fix atmospheric nitrogen. To test this point further, a series of culture flasks each containing 50 c.c. of Ashby's solution and a gram of sterilised rice soil was prepared. Nine rice seedlings were grown in each of the culture flasks for periods varying from ten minutes to ten days and the flasks were subsequently inoculated with Is. III. The contents of the culture flasks were analysed for total nitrogen at the end of twenty days after the inoculation of each flask. The results are given in Table IV.

TABLE IV

Effect of growing rice seedlings in culture previous to inoculation on nitrogen-fixing power of the organism

Period for which rice seedlings grown previous to inoculation	*Total nitrogen in mg.	
	Individual flask	Average
10 min.	4·990 5·122	5·056
30 min.	4·294 4·442	4·368
3 hours	4·016 3·864	3·940
1 day	3·300 3·378	3·339
3 days	2·784 2·848	2·816
7 days	2·984 2·848	2·916
10 days	2·388 2·432	2·410
Control (with Is. III)	2·499 2·499	2·499

* Exclusive of nitrogen in nine rice seedlings removed previous to inoculation.

It is clear from the table that progressively less amounts of nitrogen are fixed by the organism in culture medium in which rice seedlings have previously been grown for ten minutes to ten days, although there is not much difference in the nitrogen contents of the culture flasks in which rice seedlings have been grown for three and seven days before inoculation. The growth of rice seedlings in culture flasks for ten days so changes the culture medium that it has a depressant effect on the organism which fixes less nitrogen than in the control. In other cases the association of the organism with the living roots stimulates its activity to a greater or less degree as seen from the amounts of nitrogen fixed.

A possible explanation of these results might be sought in a change in the reaction of the culture medium brought about by rice seedlings growing in it for varying lengths of time. The above experiment was therefore repeated with some modifications in the length of time for which the rice seedlings were grown in the culture medium. The reactions of the solution in each culture flask were determined electrometrically at the beginning and at the conclusion of the experiment. The results in Table V indicate that the solution becomes more alkaline as the period of contact of living rice roots with Ashby's solution increases.

TABLE V

Reaction of Ashby's solution in which rice seedlings were grown for varying lengths of time

Period for which rice seedlings grown previous to inoculation	*p*H	
	Initial	After removal of plants
10 min.	6·88	6·90
30 min.	6·88	7·16
3 hours	6·88	7·17
2 days	6·88	7·19
5 days	6·88	7·50
7 days	6·88	7·64
10 days	6·88	7·90

Experiment V.—This experiment was done to ascertain the *p*H of the culture medium favouring the most vigorous development of the organism. Ashby's solution (without calcium carbonate), to which one gram of sterilised rice soil was added, was adjusted to different *p*H by adding to it appropriate

quantities of $N/100$ hydrochloric acid and $N/100$ caustic soda to give a range of pH varying from 4·79 to 7·59 after the sterilisation of the medium. The culture flasks were subsequently inoculated with Is. III and incubated at room temperature for 20 days. The pH of the solution in each culture flask was determined at the conclusion of the experiment and also the amount of nitrogen fixed by the organism. The results are recorded in Table VI.

TABLE VI

Effect of different p*H on the development of the organism*

Initial pH	pH at conclusion of experiment	Total nitrogen in mg.
4·79	5·38	1·606
5·63	5·50	2·047
5·67	5·66	2·031
6·06	5·75	2·912
6·30	5·78	3·679
6·54	5·84	3·533
6·79	6·01	3·109
6·91	6·32	3·106
7·20	6·55	3·505
7·29	6·58	3·128
7·59	6·63	3·185
6·06 (Control)	5·84	0·975

The results show that the organism fixes the largest amounts of nitrogen at pH 6·3 to 6·54 ; on either side of this range the nitrogen-fixing power of the organism decreases although it is more adversely affected in the acidic than in the alkaline medium. The organism is able to produce acid by the utilisation of mannite in the medium as shown by the more acid reaction in almost every case at the conclusion of the experiment. This evidence, when considered with the data in Tables IV and V, clearly establishes the fact that the organism is most active in a slightly acid medium and, as the medium is rendered more alkaline by contact with growing rice seedlings, the activity of the organism to fix nitrogen suffers a decline.

Organism

The organism is a species of *Azotobacter*, and the following is a description of its morphology and cultural and physiological characteristics :—

Rods : 2·0 to 3·2 by 2·0 to 2·8 μ, occurring in pairs (diplococcus) and a few in packets (pediococcus). Motile. Encapsulated. Gram-negative. Brown to black pigment begins from the third day onwards. Pigment production begins earlier in thickly seeded plates, colonies buried in the medium not producing pigment.

Frazier's gelatin medium : No liquefaction.

Ashby's mannitol agar colonies : Glistening, opaque, circular, raised, smooth.

Neutral beef broth : No growth.

Litmus milk : Becomes clearer in seven to fourteen days.

Potato cylinders : Moderate, glistening, brown, slightly slimy growth.

The organism fixes atmospheric nitrogen and grows in a crude, black film on the surface of Ashby's mannitol solution ; it produces slight acid but no gas when mannitol in Ashby's solution is replaced by saccharine, maltose, galactose, levulose, lactose, raffinose, glucose, salicin, dextrin, asparagin, amygdalin, glycerine and inulin.

Aerobic.

Optimum temperature about 26°C.

Habitat : Soil.

Periodicity

The main purpose of this study was to estimate the density of *Azotobacter*-like organisms in rice soils at Karjat in order to ascertain roughly the numbers occurring in any one year as part of a normal recurring cycle. The seasonal changes in numbers of the organism have an important bearing on factors affecting crop yield, and it was held to be probable that there existed a correlation between the numbers of non-symbiotic organisms of the type of *Azotobacter* in cultivated rice soils at Karjat and the nitrogen-content of the latter.

Beginning with August, composite samples were taken from the 2-4 in. layer of cultivated rice soil at Karjat and of fallow at intervals of about three weeks during 1936-37. Plate cultures of different dilutions were prepared from one-gram samples of soils, and a selective medium like Ashby's agar was used for culturing the organism. The relative incidence of different types of organisms was noted by an examination of the colonies, and the number of such bacterial colonies on a plate as resembled *Azotobacter* colonies was counted. In each culture flask the determination of total nitrogen including nitric nitrogen was done according to the method described in the " Official and Tentative Methods of Analysis " by the Association of Official Agricultural Chemists [1935]. For each determination five grams of dry

soil were taken and wetted with water previous to the addition of salicylic-sulphuric acid mixture. The results are recorded in Table VII and those for cultivated soil are also shown graphically in Fig. 1.

TABLE VII

Azotobacter-*like organisms in rice soil and fallow per gram*

Date of collection	Cultivated soil		Fallow	
	No. of colonies	Total nitrogen in mg.	No. of colonies	Total nitrogen in mg.
August 3, 1936 . . .	1,600,000	0·837	0	0·686
August 27, 1936 . . .	340,000	1·024	0	0·668
September 23, 1936 . .	56,000	1·170	0	0·674
October 10, 1936 . . .	41,000	0·930	0	0·718
October 27, 1936 . . .	2,000	1·009	0	0·780
November 21, 1936 . .	300	0·964	0	0·612
December 12, 1936 . .	1,000	0·989	0	0·674
January 2, 1937 . . .	0	0·710	0	0·892
January 30, 1937 . . .	0	0·776	0	0·729
February 21, 1937. . .	300	0·772	0	0·531
March 15, 1937 . . .	0	0·752	0	0·522
April 5, 1937 . . .	450	0·801	0	0·774
April 27, 1937 . . .	25	0·807	0	0·450
May 8, 1937 . . .	0	0·726	0	0·549
May 29, 1937 . . .	500	0·773	0	0·994
June 19, 1937 . . .	400	0·872	0	0·846
July 10, 1937 . . .	8,000	0·910	0	0·687
July 31, 1937 . . .	110,000	0·815	0	0·619
August 21, 1937 . . .	280,000	0·729	0	0·483

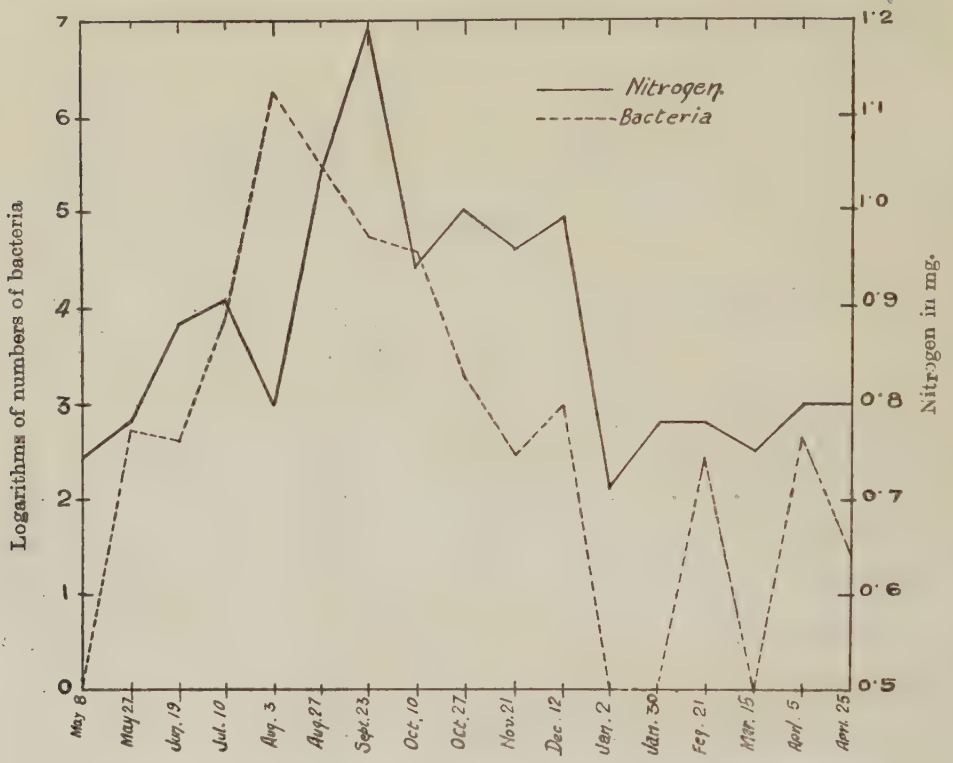

Fig. 1 Density of *Azotobacter* in and nitrogen content of rice soil in different seasons.

It will be seen from Table VII that fluctuations in the density of *Azotobacter*-like organisms in the rice soils are of such magnitude that numbers of these bacteria found in any one month cannot be accepted as representing the actual state of flora during other times of the year. These fluctuations are due to external climatic influences in the soil since the organism is very abundant during the rainy season from July to October when the rice crop is growing. After the monsoon the numbers of bacteria suffer a sharp decline until the organism becomes scarce and almost disappears from the soil in the cold season and during hot months. These seasonal fluctuations in microbial activity, though apparently due to climatic influences, may also have something to do with the growth of the crop since fallow does not support any population of *Azotobacter*-like organisms. The microbial activity in cropped soils is further reflected in an increase in the nitrogen-content of these soils as compared with fallow, more particularly in the rainy season. The presence of straw and its derivatives and large amounts of other organic matter as sources of energy for nitrogen-fixing bacteria will explain the great predominance of these organisms in cultivated rice soils and their complete absence from fallow lands.

Summary

It has been shown that *Azotobacter* plays an important role in the nitrogen recuperation of rice soils at Karjat. The organism is stimulated to greater activity when associated with the growing roots of rice plants, which seem to cause an increase in its efficiency of nitrogen fixation by bringing about a change in the reaction of the medium in the vicinity of living roots.

The organism shows a marked periodicity due probably to external climatic influences in the soil as it is very abundant during the monsoon but suffers a sharp decline in numbers in the dry period following the rains until it becomes scarce and almost disappears from the soil in the cold season and during hot months. This microbial activity during the monsoon is reflected in an increase in the nitrogen-content of the rice soils. The seasonal fluctuations in microbial activity may also have something to do with the growth of the crop since fallow does not support any population of *Azotobacter*-like organisms.

Acknowledgment

In conclusion the writers wish to acknowledge the assistance rendered by Dr V. N. Gokhale and Mr. M. M. Kibe in analysing samples for their nitrogen-content.

Addendum

After this paper was submitted for publication, the present writers saw a note on " Nitrogen fixation by blue-green algae " by F. E. Fritsch and P. K. De [*Nature* **142**, 878. 1938] in which the latter state that they have isolated in pure cultures three species of *Anabaena* which have the property of fixing considerable quantities of nitrogen from the air. There was, however, no increase in the amount of nitrogen fixed when mixed cultures of *Anabaena* and *Azotobacter* were used, from which the authors conclude that *Azotobacter* plays " a relatively unimportant part in nitrogen fixation in the rice fields "— a fact which is contrary to the results reported in the present paper in so far as the soils of the rice fields at Karjat are concerned.

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THE STEM-BLACK DISEASE OF APPLE IN KUMAUN

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(With Plates XXXII and XXXIII)

Introduction

The stem-black disease of apple is caused by *Coniothecium chomatos porum* Corda. Strains of this fungus were found by Shaw [1918] and Butler [1919] to cause apple-cracking and branch blisters of apple trees in Kumaun. A similar stem-black disease of apple, though differing in symptoms from the one occurring in Kumaun, is described by G. H. Cunningham [1907] in his book ' Fungus Diseases of Fruit Trees in New Zealand '. It was first recorded there by Kirk [1907]. The disease is known in Australia, England and South Africa and in India it is known to occur in Baluchistan, Kurram Valley, Solan (Khattoo Fruit Orchards) and Kulu. It is now found in every orchard in Kumaun. The causal fungus usually attacks and kills the thick branches of apple trees. The death of a thick branch means considerably less apple crop and since the disease is present in all the orchards in Kumaun the economic loss due to it to the orchardists must be very considerable. A survey in the Government Orchards, Chaubattia in Kumaun, in 1936, showed that 60 per cent of the apple trees had stem-black. The death of the affected branches is hastened sometimes by the attack of *Botryosphaeria ribis*, the causal fungus of the stem-brown disease of apple. Other fruit trees are not known to be affected by the stem-black. The disease appears by the middle of July and is in its maximum virulence by the middle of August.

Symptoms

The disease starts at the pruned surfaces and proceeds downwards, forming a jet black streak, which slowly extends, surrounds the whole of the branch and ultimately kills it (Plate XXXII, figs. 1 and 2). In advanced stages cankers are formed (Plate XXXII, fig. 3). Thick branches are more susceptible than thin ones. The fungus was not observed to attack uninjured surface.

Morphology

An examination of a large number of transverse sections of the diseased portions showed that the fungus killed the tissue of the cortex down to the wood (Plate XXXIII, fig. 1). The hyphae were very short and were hardly different from the conidia. The conidia were not catenate ; they were irregular circiniform, muticate, often coalescent. The conidiophores and conidia were dark brown in colour (Plate XXXIII, figs. 2, 2*a* and 2*b*). The conidia measured $8 \cdot 4 - 36 \cdot 4\mu \times 5 \cdot 6 - 30 \cdot 8\mu$, the average being $16 \cdot 3\mu \times 11 \cdot 4\mu$. Their size was, thus, very variable. Chlamydospores were formed in nature (Plate XXXIII, fig. 4). Both conidia and chlamydospores germinated readily in tap water (Plate XXXIII, figs. 3 and 4*a*) within 24 hours. It was observed that about 5 per cent of them grew directly into germ tubes, while the rest burst and released a large number of minute, hyaline secondary spores. The latter, after a time, either produced buds or grew into germ tubes. The buds also germinated and produced germ tubes (Plate XXXIII, figs. 5 and 6). The secondary spores measured from $2 \cdot 8 - 7 \cdot 0\mu \times 2 \cdot 8 - 6 \cdot 3\mu$, the average being $4 \cdot 79\mu \times 4 \cdot 08\mu$ (Plate XXXIII, fig. 5). Rupturing of the conidia into secondary spores and their budding were also observed by Massee [1915] and he considered the secondary spores to be the potent source of fresh infection.

Isolation

The isolation of the causal fungus was made with single spores from diseased stems. The fungus was black in colour and very slow growing.

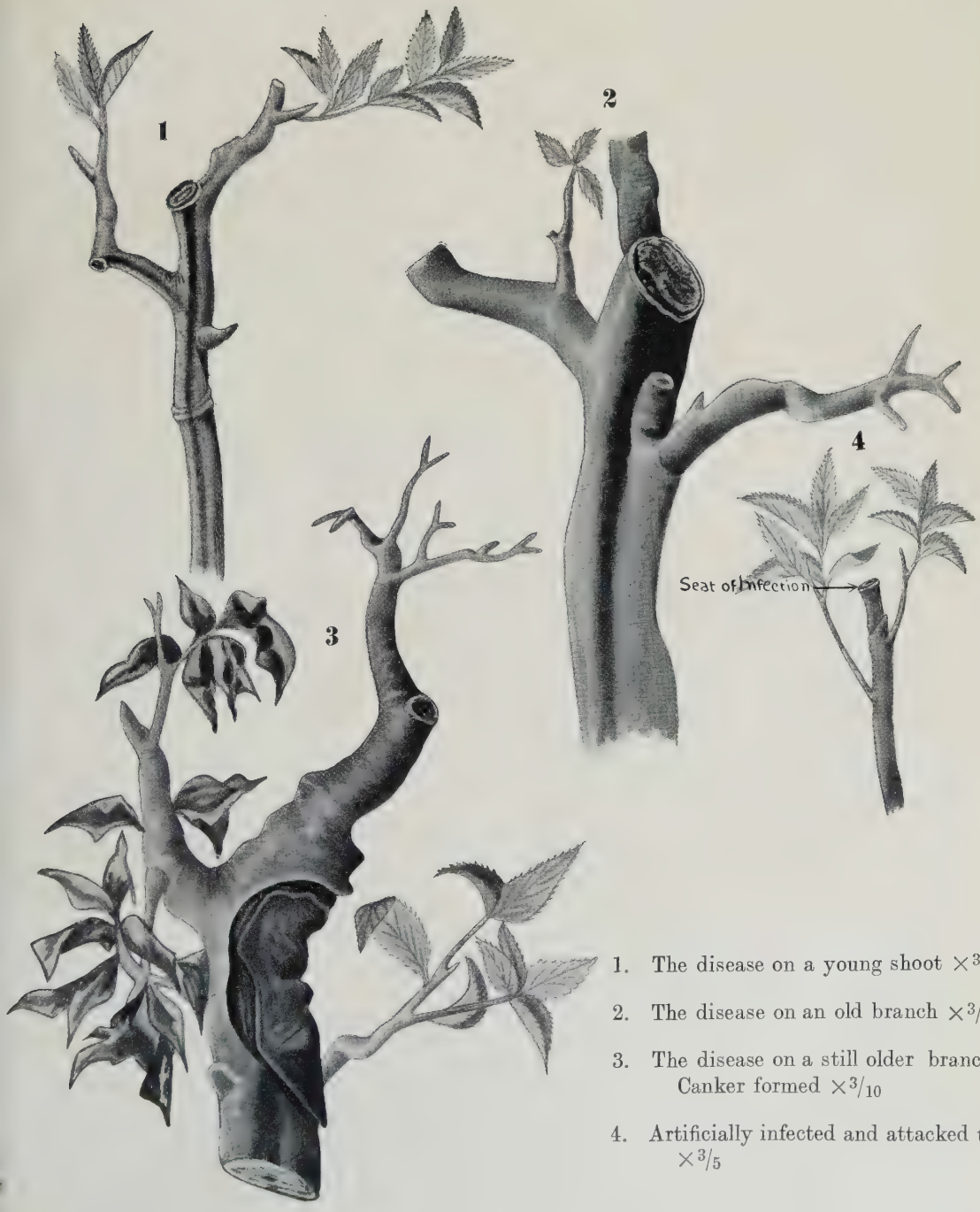
Pathogenicity studies

(*a*) A number of infection experiments were carried out by placing drops of spore suspension on uninjured surfaces of stem ; but in no case infection took place. When the surface was injured, however, the fungus readily penetrated and ramified in the tissues.

(*b*) On the 5th July 1935, three one-year old plants of apple, variety Delicious, were pruned and the cut surfaces were inoculated with the pure culture of the fungus. They were then covered with sterilised wet absorbent cotton and enclosed in cellophane bags. Two other one-year old plants of the same variety were similarly treated but were not inoculated. They served as controls. All the plants were kept inside a glass cage in which water was sprayed from time to time to maintain a saturated atmosphere. After seven days the cellophane bags were removed. Infection was noticed to have taken place in all the inoculated surfaces ; the controls remained unchanged.

Another experiment was similarly started on the 13th July with three plants of the variety Esopus Spitzenberg. The infection took place in all of them within seven days, except in the controls.

STEM-BLACK DISEASE OF APPLE CAUSED BY *CONIOTHECIUM CHOMATOSPORUM* CORDA



1. The disease on a young shoot $\times 3/10$
2. The disease on an old branch $\times 3/10$
3. The disease on a still older branch. Canker formed $\times 3/10$
4. Artificially infected and attacked twig. $\times 3/5$

2
3
4
4a
2a
1
2b
7a
7
6
7b
5

PLATE XXXIII

FIG. 1. The transverse section, 6 μ thick of the bark of the apple stem showing the affected portion (×480)
FIG. 2. Conidia of the causal fungus *Coniothecium chomatosporum* (×480)
FIG. 2*a* and 2*b*. Conidiophores with conidia (×480)
FIG. 3. Germinating conidia (×480)
FIG. 4. Chlamydospores (×480)
FIG. 4*a*. Germinating chlamydospores (×480)
FIG. 5. A ruptured conidium with the released secondary spores. Some of the latter are budding (×480)
FIG. 6. Germinating secondary conidia (×480)
FIG. 7, 7*a* and 7*b*. Mycelium and chlamydospores in culture (×480)

A third experiment was carried out with six seedling apple plants on the 5th of May of the following year. Two of them served as controls. Infection took place in ten days in all the inoculated plants. The first symptom of infection was the collapse of the tissue and the consequent reduction of the diameter of the infected portion. Its colour became light brown, gradually changing to dark brown, and finally to sooty black (Plate XXXIII, fig. 4). Numerous small blisters appeared on the surface, which later on burst into distinct longitudinal cracks. Innumerable conidia of *Coniothecium* could be seen with the lens all over the infected area.

The fungus was re-isolated in every case and resembled the parent culture in all respects.

Cultural studies

An extensive study of the behaviour of the fungus in oatmeal agar, Brown's starch synthetic agar and Czapek's agar was carried out. Cultures were made in Petri dishes of uniform size of 9 cm. diameter, each containing 10 c.c. of the medium. A small bit of hypha was planted in the centre of each dish. The inoculated dishes were kept in the room where the temperature varied from the maximum of 73°-87°F. to the minimum of 62°-71°F. The growth of the colonies was measured at intervals of four days up to 20 days and the macroscopic and microscopic characters were noted after a month. Conidia were never produced in these media.

Table I

Measurements of the linear growth of the colonies of Coniothecium chomatosporum *in three different media*

	Average diameter of the colonies				
	4th day	8th day	12th day	16th day	20th day
	mm.	mm.	mm.	mm.	mm.
Oatmeal agar	7·50	12·76	17·68	24·18	27·56
Czapek's agar	8·07	14·35	20·25	24·0	25·0
Brown's starch synthetic agar	7·20	19·91	18·75	25·5	29·0

In addition to these three media the fungus was also grown on potato-dextrose, Brown's synthetic, apple stem decoction and Coon's agar media and also on sterilised apple stems and leaves, to see if conidia formation could be induced. But they were not formed in these media also. In all

the media tried only the chlamydospores appeared (Plate XXXIII, fig. 4). The mycelium was in every case thick, light to dark brown in colour, highly granular and closely septate. There were faint and narrow zonations in the cultures.

The fungus was very slow growing in all the media. Its growth was inhibited at the temperature of 86°F. but it was resumed when the temperature was lowered.

Sources of infection

In New Zealand, where this fungus is known to attack apple stem and fruits, two spore-stages are found. The *Coniothecium* stage, which is parasitic, and a second stage, which is apparently saprophytic occurring on dead bark of old diseased areas. Until recently, only the *Coniothecium* stage was known. But Massee [1915] found a pycnidial or *Phoma* stage associated with *Coniothecium*, and he and Van der Bijl [1915], later on, were able definitely to link them together. In New Zealand the fungus overwinters on shoots either in the form of pycnidia, or in the tissues as mycelium of the *Coniothecium* stage. In Kumaun the pycnidial stage is not met with and the fungus overwinters in the mycelial form in the shoots of cankered branches. The primary conidia of *Coniothecium* seems to be the chief agent for the spread of the disease. The minute hyaline spores, produced by budding from the secondary spores originating from the primary conidia, may also serve as a source of infection.

The disease usually starts in July and the infection is the highest by the middle of August. Innumerable primary conidia and secondary spores were readily caught, towards the middle of August, on vaseline-smeared slides exposed in spore traps (aeroscopes). The disease can thus be held to be carried by wind. But it can also spread through rain water. Drops of water collected from cankered areas after a shower of rain showed numerous spores of the fungus in them. The pruned surfaces below a diseased area very frequently become infected, probably through contaminated drops of rain water. Whether they are carried by insects has not been determined.

Control

Since the mycelium remains imbedded in the tissue of the host, spraying or dusting will have no effect in controlling the disease. Butler [1919] observed that spraying with lime, sulphur and Burgundy mixture failed to check it completely. Careful pruning of the affected areas and branches appears to be the only means of getting rid of the disease where it is established. But to prevent fresh infection a paint or smear, which can well cover the cut surfaces of the branches and at the same time stop germination of the spores of the fungus settling on them, can be the only means.

With this point in view an experiment was laid out in 1936 to try the efficacy of pastes made with 100 c.c. raw linseed oil and each of the following ingredients and of Ostico banding grease :—

	oz.
Dried precipitate of Bordeaux mixture . .	4
Copper carbonate	4
Red lead powder	8
Red lead powder	2
Copper carbonate	2

The total number of treatments along with the controls in this experiment was seven, *viz.*, (1) untreated and uninoculated (control), (2) untreated and inoculated, (3) Bordeaux paste, (4) copper carbonate paste, (5) red lead paste, (6) red lead and copper carbonate paste and (7) " Ostico " banding grease. All these treatments were given on pruned surfaces on each of twelve old apple trees selected at random in different blocks of the orchard. Each treatment was replicated twelve times. The pastes were applied on the cut surfaces from the 14th to 18th July 1936, and barring the controls, all of them were inoculated with bits of the culture of *Coniothecium chomatosporum* from the 29th July to 2nd August 1936. This culture was growing on Brown's starch synthetic agar medium at room temperature for 15 days. After inoculation each surface was covered with a piece of sterilised wet cotton wool and enclosed in cellophane bags in order to maintain the moisture condition necessary for infection. The cellophane bags were removed after a fortnight. Infection was quite visible in every one of the untreated and inoculated surfaces and also in some of the treated ones. The experimental twigs were cut and brought into the laboratory for microscopic examination.

Table II gives the results of the analysis of variance and the comparisons of the mean percentage infection for the different treatments.

TABLE II

(*a*) *Analysis of variance*

——	Degree of freedom	Sum of squares	Mean square	" F "
Blocks	11	4941·7348	449·2486	7·70
Treatments	6	42517·6868	7086·2611	*121·43
Error	66	3851·5820	58·3570	

* Highly significant.

(*b*) *Summary of results*

—	Treatments							Standard error of difference
	1	2	3	4	5	6	7	
Mean percentage infection.	100	100	83	86	74	31	100	3·11

Conclusions :—(1)=(2)=(7)>(4)=(3)>(5)>6.

It is clear from these tables that the paste made by mixing 2 oz. of red lead and 2 oz. of copper carbonate with 100 c.c. of raw linseed oil is found to be most effective in controlling fresh infection of the disease.

In 1937 the following treatments were, in addition, tried against painting with the paste made of red lead, copper carbonate and raw linseed oil to discover any other paste which would be equal to or better than the latter.—

(1) Painting with the paste prepared by mixing 2 oz. of red lead and 2 oz. of dried precipitate of Bordeaux mixture in 100 c.c. of raw linseed oil

(2) Washing the cut surfaces with 1-1000 $HgCl_2$ previous to painting with Bordeaux paste prepared by mixing 4 oz. of dried Bordeaux mixture with 100 c.c. of raw linseed oil

(3) Painting with a paste of linseed oil 100 c.c., and lime sulphur 4 oz. (equal quantities of lime and sulphur were self boiled before adding linseed oil).

The experiment was laid out in exactly the same way as that of 1936.

Table III gives the results of the analysis of variance and the comparisons of the mean percentage infections for the different treatments.

TABLE III

(*a*) *Analysis of variance*

—	Degree of freedom	Sum of squares	Means quare	"F"
Block	11	3221·093	292·826	2·28
Treatment	5	85760·7048	17132·1409	*133·51
Error	55	7057·6592	128·321	

*Highly significant.

(b) Summary of results

	Treatments						
	Untreated (uninoculated)	Untreated (inoculated)	Red lead + copper carbonate paste	Red lead + Bordeaux paste	$HgCl_2$ followed by Bordeaux paste	Lime sulphur paste	Standard error of difference
	1	2	3	4	5	6	
Mean percentage infection.	100	100	8	32	66	84	4·6

Conclusions :—(1) = (2) > (6) > (5) > (4) > (3)

It is, therefore, proved that the paste prepared with equal quantities of red lead and copper carbonate in raw linseed oil was the best of all the pastes tried in preventing fresh infection by *Coniothecium chomatosporum*.

Summary

1. The stem-black disease of apple, caused by *Coniothecium chomatosporum* Corda, occurs extensively in every orchard in Kumaun.

2. The fungus infects pruned surfaces and proceeds downwards, rendering the affected areas jet black. Cankers are formed and the branches are ultimately killed. Thick branches are more susceptible than thin ones. The tissues are killed up to the cambium and the fungus hibernates in the tissues.

3. The conidia are dark brown, circiniform, muticate, often coalescent and are very variable in size. Some of the conidia burst and give rise to secondary hyaline spores. The secondary spores either grow into germ tubes or produce buds. The buds on their part also grow into germ tubes.

4. The spores are carried by wind and with rain water.

5. The fungus cannot enter through uninjured surface of the stem but can do so only through wounds.

6. A detailed cultural study of the fungus is made. The fungus is slow growing and does not produce conidia in any of the media tried. Only chlamydospores are formed.

7. Painting of the pruned surfaces with a paste made of 2 oz. each of red lead and copper carbonate and 100 c.c. of raw linseed oil very effectively controlled the spread of the disease.

Acknowledgment

The writers are deeply indebted to the Imperial Council of Agricultural Research, for the grant of funds for the Hill Fruit Research Scheme of the United Provinces, with the help of which the work was carried out at the Government Fruit Research Station, Chaubattia, United Provinces. They are also grateful to the Statistician of the Council for scrutinising Tables II and III and re-arranging them in their present form.

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A FRUIT ROT OF APPLES CAUSED BY A SPECIES OF *RHIZOPUS*

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(With Plate XXXIV and two text-figures)

Introduction

Very few fungi associated with " rots " of ripe apple fruits are recorded in India and probably none on unripe fruits. In the United Provinces [Dey, 1933] it has been found that *Aspergillus niger* Van. Tieghem is the common organism causing soft rot of apple. In other countries, on the other hand, many fungi are known to damage apples in storage [Rose, 1924].

In the course of investigations on codling moth of apples in Quetta, the samples sent to the Imperial Entomologist were often received in a rotting condition. In many cases such fruits were also observed on the trees. These samples were sent throughout the months of June and July 1937 and consisted of fruits in different stages of maturity, but usually they were unripe. In most cases (over a hundred fruits examined) the freshly rotted portions gave a pure culture of a species of *Rhizopus*. Some fruits, especially those approaching maturity, showed *Aspergillus niger* on the rotted portion. It was rather noticeable that in such fruits a species of *Rhizopus* did not occur although the samples of fruits rotted by the two fungi were packed in the same lot.

Preliminary experiments showed that the species of *Rhizopus* was a vigorous wound parasite, capable of attacking the ripe and green apples alike*. It has not yet been possible to ascertain the amount of damage due to this fungus but on two occasions rotted apples purchased early in the season (August) from the local market revealed the presence of a similar species of *Rhizopus*.

In 1938 a further sample was received, this time from Kurram Valley. There was no sign of insect attack. In a letter sent by the Extra Assistant Director of Agriculture, Kurram Valley, it was stated that the apples first changed from green to yellow and then pink before becoming brown. Rotting was confined to the stem-end, and was found to be caused by a species of *Rhizopus* similar to that found in the previous year.

*A preliminary account of this work has appeared in *Current Science*, **6**, 58-9, 1937.

Symptoms, morbid anatomy and morphology

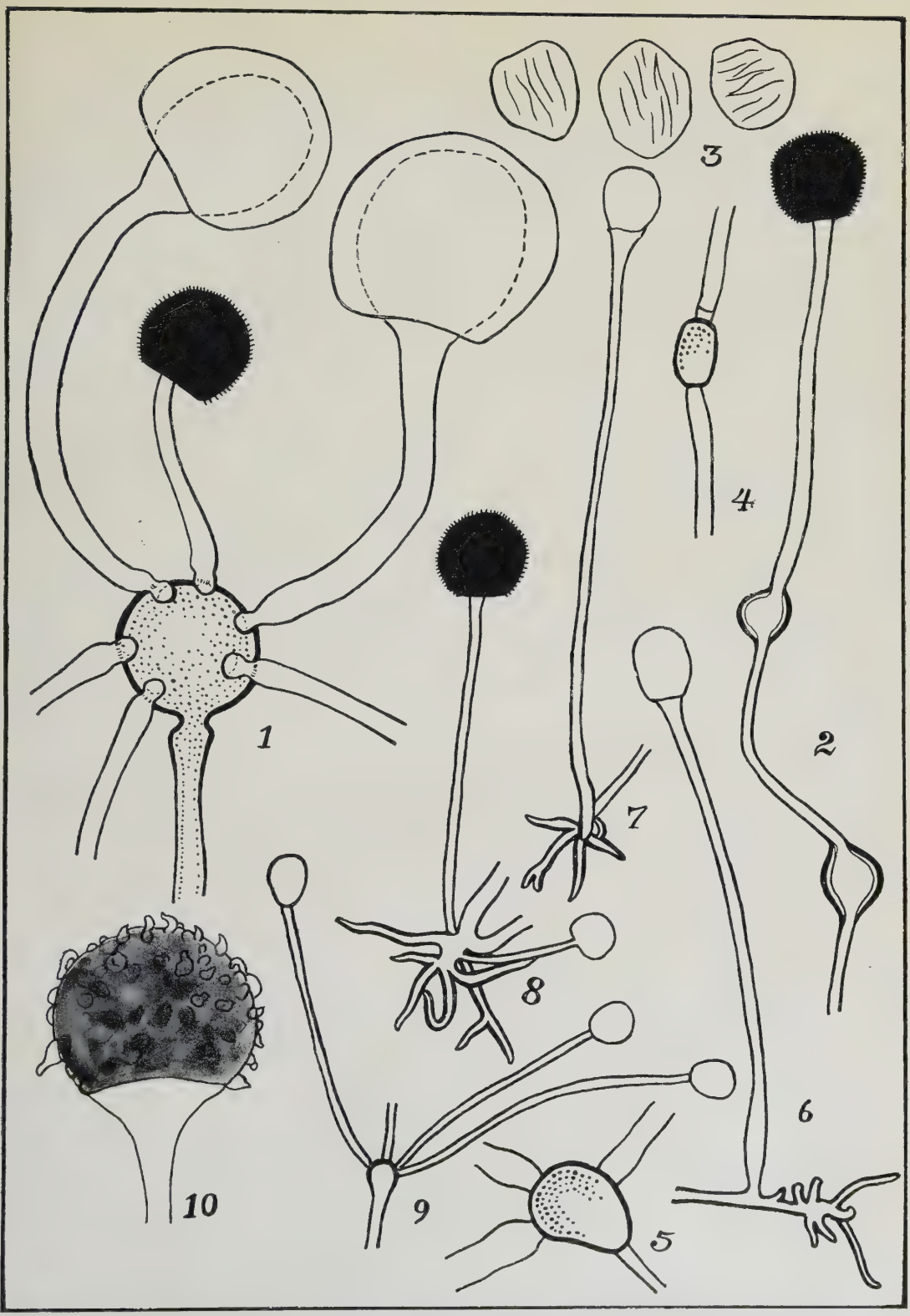
Symptoms.—The rotting fruits in the earlier stages did not show any exudation of liquid. In the advanced stages of decay the skin on the whole surface was thrown into wrinkles and a liquid exuded. There was no progressive sinking of the originally infected patch nor was the surface moist and shiny as noted by Dey [1933] in the fruits affected by *A. niger*. On the " Kulu " variety of green apples (flat, yellow-skinned fruits) the skin of the affected portions became " russet " coloured or " verona-brown " [Ridgway, 1912] and could be easily peeled away from the underlying tissue. The latter was discoloured to zinc-orange. The fruits had a slightly sour but not unpleasant odour. Since the core hardly offered any resistance, they were easy to cut across. The scales within which the seeds were enclosed easily became detached from the tissue of the core.

There was no fungus growth on the surface of the fruits but on cutting them open it was noticed that the seeds were covered with a weft of mycelium studded with black bodies. In certain cases the seeds were absent but a wad of mycelium was present in that location. Whenever the skin was removed or the fruit cut open, whitish aerial mycelium appeared on the surface within 24 hours.

Morbid anatomy.—Microscopic examination of the pulp showed the presence of broad (12-16 μ), coarse, aseptate light brown hyphae following a tortuous course, probably due to their being intercellular. These main hyphae produced numerous short, narrow, lateral branches which usually penetrated the host cells and sometimes the intercellular spaces also. The host cells abutting on the main hyphae were darker brown than those further away and these hyphae could, therefore, be easily traced. In the green apples there was no killing in advance ; the rotting was closely associated with the presence of hyphae.

Morphology.—The morphology of the fungus on the host and on culture medium was studied. The fungus was isolated from the diseased tissues and from the fungus growth on the seeds. The macroscopic and microscopic growth characters of the different isolations were compared on agar medium and found to be identical. Eventually single spore isolations from these cultures were made.

The mycelium occurring on the seed and within the scales containing the seeds was composed of broad (12-20μ) aseptate hyphae with a few isolated, intercalary hyaline chlamydospores (20 × 14μ). Rhizoids were rare. The sporangiophores were borne on bulbous brownish swellings 40-80μ in diameter of the hyphae (Plate XXXIV, figs. 1 and 2), or were terminations of ordinary hyphae. On these bulbous swellings one to many brownish sporangiophores (usually 1 to 3) were produced in the manner figured for *R. arrhizus* Fischer [Zycha, 1935] and for *R. nodosus* Nym. [Lendner, 1908]. These sporangiophores measured 160-480 μ in length and 10-12 μ in width. The sporangia were dark, more or less globose, (Plate XXXIV, fig. 1), 80-176 μ in diameter



1. A group of sporangiophores arising from the brown bulbous swelling. Taken from host (× 300). 2. One sporangiophore and two bulbous swellings on the hyphae. Taken from host (× 160). 3. Spores (× 1800). 4. Intercalary chlamydospore (× 300). 5. Intercalary chlamydospore (× 300). 6—9. Different sizes and manners in which sporangia are produced on malt agar (× 160). 10. A deformed sporangium. Taken from host (× 300).

mean 114 μ (100 measurements). The columella was more or less oval, 60-100 × 66-112 μ with a mean diameter of 88 × 96 μ. A small percentage of deformed sporangia were also sometimes noted. In these the contents instead of differentiating into spores developed into numerous small, hyaline chlamydospore-like bodies with a very characteristic shape (Plate XXXIV, fig. 10). Such sporangia have been noted previously [Yamamoto, 1930] to occur commonly in the *Rhizopus albus* group.

The spores were light grey, plain-surfaced, slightly angular (nearly polyhedral), faintly striated and measured 5-7·2 μ, with a mean of 7·0 μ (200 measurements).

Good aerial growth, which was first white but later light brownish, occurred on oat agar, malt agar (3 per cent), potato-dextrose agar, and apple extract agar, but it was poor on Hopkins' and Dox's agar. On these media light to dark brown rhizoids developed but their formation was poor on Hopkins', Dox's and apple extract agar. Their formation was not correlated with the amount of aerial growth, for on apple extract agar medium and cut apples aerial growth was copious but rhizoids were scanty. The stolons were hyaline except for the small length near the rhizoids. Hyaline chlamydospores developed in stolons as well as in the ordinary hyphae. They were formed close to a septum (Plate XXXIV, fig. 4).

Sporangiophores and sporangia were copiously formed on most of the media. The sporangiophores arose on the nodes (rhizoids), on the stolons and from the ordinary hyphae (Plate XXXIV, figs., 6-9).

In cultures a great variation in the size and shape of the sporangia, depending on their position, was noted. Those which were produced on the stolons and nodes conformed to the size and description given previously ; those which developed on the aerial hyphae, on the other hand, were often much smaller, more flat (sub-hemispherical) and their columella was ellipsoidal. The bulbous swellings on hyphae, mentioned previously, were rather infrequent in cultures, and when they did appear it was more commonly in culture tubes than in Petri-dish cultures.

Abnormal sporangia were rare on some media and very frequent on others. Yamamoto [1930] has emphasised this character in the key for the classification of the species of *Rhizopus*. The studies made on this particular fungus, suggest that this is not a very stable character. These abnormal sporangia were common on apple extract agar (*p*H 5·2). Their contents broke up, sometimes, into a very few big segments (4 to 8), but more commonly, however, into numerous hyaline beaked or curved bodies. Sometimes a part of the protoplasm of the sporangium developed normal spores and the rest into the above mentioned bodies. Direct germination of the sporangium was also noted but very rarely. These abnormal sporangia tended to be more flat and brownish.

The size and shape of the sporangium did not seem to be affected by temperature. The striations on the spores, however, looked more prominent at 26°C, than at 15° or 32°C.

Infection experiments

Preliminary infection experiments were made on a few varieties of ripe apples available in the local market. Fruits were surface sterilised and sporulating mycelium was placed on the surface which was then gently pricked with a sterile needle to injure the skin. These apples were placed in sterile, moist chambers at the room temperature (96-100°F). Definite spots were found (0·5 to 2 cm. in diameter) in all the inoculated apples within 24 hours while the controls remained unaffected. Out of 21 inoculations 20 were successful. The size of the spot varied from apple to apple, even on the same variety. In the purple-skinned varieties there was an intensification of the colour at the margin of the spot. The depth of rotting increased at a rate equal to the increase in radius of the spot at the surface. Examination of completely decayed apples showed that the fungus had enveloped the seed with a weft of mycelium just as in a naturally infected fruit.

In order to carry out the infection experiments critically, semi-mature green-skinned fruits (slightly sweet to the taste) of one variety and age were obtained from Quetta. The method of inoculation used was that of Granger and Horne [1924]. All the apples used in the experiment were first placed for one day at 35°C. to see that they carried no previous infection. Each apple was then weighed. With a sterile cork borer a cylinder of tissue was removed to a depth of half an inch. A thick spore suspension (about 0·1 c.c.) was introduced with a sterilised pipette, the plug was replaced, and the wound was sealed with paraffin. The apple was wrapped in tissue paper and placed at the required temperature. At the expiration of the desired time it was removed, the rotten tissue was scraped away, and the healthy tissue was weighed. The experiment was conducted in triplicate at temperatures between 32°C. and 40°C. and in duplicate for lower temperatures. The effect of temperature on the percentage of rotting of green fruits is shown in Table I.

Table I

Effect of temperature on the percentage apple tissue rotted

Period of incubation (hours)	Percentage of rotting				
	24°C.	32°C.	35°C.	37°C.	40°C.
24	0·8	3·6	5·2	7·0	8·5
48	2·2	14·2	19·6	26·5	20·1
72	6·8	56·0	81·4	100·0	100·0
96	12·6	94·4	100·0	..	..
120	22·0	100·0	..	..	..
144	31·5	..	..	..	..

It will be seen from Table I that rotting occurred from 22°C. upwards and its rate was very fast from 32°C. upwards.

Effect of temperature on growth of the fungus

Experiments were also made to determine the thermal relations of the fungus on agar media. Plates of equal sizes uniformly poured were used in triplicate. Dox's, Hopkins' and malt agar media were used but, as the former two media gave very sparse, fluffy growth, they were found unsatisfactory. The fungus was very fast growing and in forty-eight hours at 35° and 37°C. it filled Petri-dishes of malt agar 11 cms. in diameter.

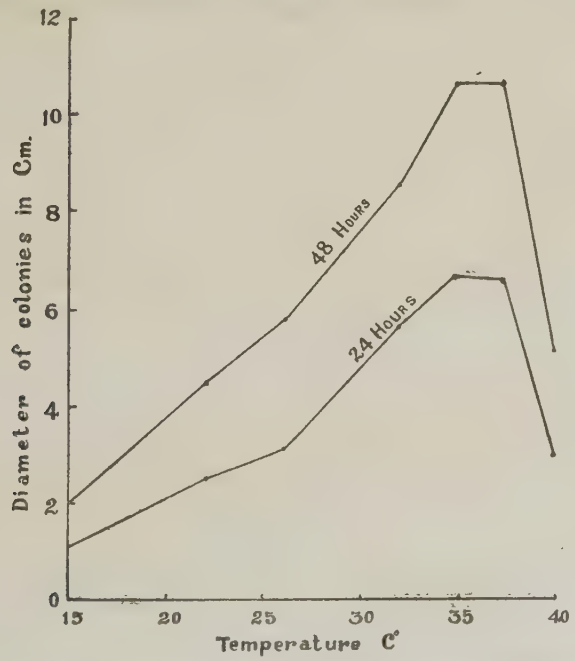


Fig. 1. Rate of growth of *Rhizopus* from apple on malt agar at different temperatures.

It can be seen from Fig. 1 that the fungus could grow from 15° to 40°C. and the optimum temperature for its growth was between 35° and 37°C. These results are in conformity with those obtained in infection experiments. It is, however, not clear why inoculated fruits were affected as seriously at 40°C. as at 35° to 37°C. Either it is due to the adverse effect of the temperature on the fruits, or to the fact that their internal temperature was lower and thus corresponded with the optimum requirements of the fungus.

Effect of reaction of medium on growth of the fungus

As stated previously, the fungus could infect both ripe and green apples which were presumbaly of different acidities. The effect of hydrogen-ion concentration on the growth of the fungus was therefore studied. Peptone

solution (2 per cent) was adjusted to different *p*H by adding varying amounts of acid and alkali according to the method of Karrer and Webb [1920]. The solutions were then inoculated by adding equal amounts of a spore suspension. After 24 hours a visible sign of growth was present in solutions in the range of *p*H from 3·0-8·4. At *p*H 3·0 and 3·2 there was a little gelatinous, submerged growth which tended to become aerial with age ; at *p*H 4·1-8·4 aerial growth was good, being most copious at *p*H 4·1 and 4·4 and decreasing progressively in more alkaline ranges. Sporangia developed from *p*H 4·1 to 8·4 but were more abundantly formed in acidic media. The fungus changed the sulphur yellow colour of the medium to orange-cinnamon at *p*H 8·4.

The dry weight of the fungus formed at different hydrogen-ion concentrations is shown in Fig. 2.

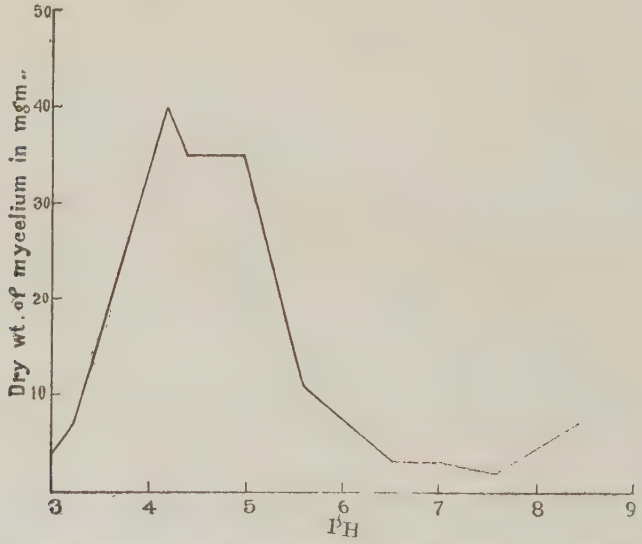


Fig. 2. Effect of hydrogen-ion concentration on the growth of *Rhizopus* from apple (after fourteen days)

The fungus tended to make the medium alkaline, a tendency especially marked in acid range. In the alkaline range it tended to be slightly more alkaline except at *p*H 8·4 where the medium became slightly acidic (*p*H 8·1).

The *p*H of the green apple was found to be 4·1 to 4·2 and the ripe apples (used in inoculation experiment) 5·2. Both these fruits, therefore, afforded a suitable substratum for the growth of the fungus, specially the green fruits. The fungus changed the *p*H of the latter from 4·1 to 5·2.

Identity of the fungus

The formation of bulbous swellings from which the sporangiophores arise has been emphasised by Lendner [1909] as a specific character of *R. nodosus*. Somewhat similar swellings were noted by Hanzawa [1912] on *R. Delemar*

(Boid.) Wehm. et Hanz. The presence of sporangiophores on swollen nodes, the scarcity of rhizoids, and the size of the spores suggest that the species under consideration is *R. arrhizus*. Zycha [1935], however, suggests that there is no valid ground for the separation of *R. arrhizus* and *R. nodosus*. He also states that both Lendner and Hagem hesitated in separating them. Yamamoto [1930,1], however, does not include *R. nodosus* in the " arrhizus group " but places in ' Oryzae group ' where the sporangia and rhizoid formation is very good and the stolons are coloured. He also separates the two species on the basis of physiological characteristics [1930,2]. He found that *R. arrhizus* formed sporangia at 15°C., but not at 41°C. while *R. nodosus* did so at both the temperatures. In the present studies sporangia developed at 15°C. and not at 41°C, while the stolons were either hyaline or pigmented to a variable distance from their point of origin on the nodes. The development of rhizoids was poor on certain media while good on others.

The species under study is assigned to *R. arrhizus* Fischer with the reservation as to its validity made by Zycha [1935] and as understood by him.

Conclusion

The parasitic potentialities of the group of the species *Rhizopus*, which are high temperature fungi, is well known in other countries and their parasitism has been established by Harter and Weimer [1922]. They state that many of these species of " intermediate temperature group " were vigorous parasites but in the United States of America none of these was met with as frequently in nature as *R. nigricans*. The presence in India of a fungus which thrives well at high temperature is not surprising.

The extent of damage to apples due to *R. arrhizus* is not yet known.

Summary

1. In the course of an investigation on the codling moth of apples in Quetta, the samples sent to the Imperial Entomologist were often received in a rotting condition. The fruits were partially ripe and had very characteristic symptoms. A further sample of fruits showing similar symptoms was received from the Kurrum Valley. Here the rotting started at the stem end while the fruits were borne on the trees.

2. The affected fruits turned yellowish brown and remained quite firm in the earlier stages of the disease. These fruits had a slightly sour but not unpleasant odour. When the fruits were cut open the seeds were invariably found embedded in a white pad of fungus mycelium. In the tissues the fungus tended to be intercellular and sent numerous short lateral branches into the cells.

3. The causal organism was identified as a species of *Rhizopus*. The mycelium occurring on the seed was composed of broad aseptate hyphae, and the rhizoids were rare. The sporangia were produced on bulbous brown swellings (1 to 3 or more on each swelling). The spores were 5-7·2 μ in diameter. Intercalary chlamydospores were present.

4. Infection experiments on ripe and unripe apples gave a very high percentage of infection. Slight wounding was necessary to start the infection. Severity of rotting was dependent on the temperature ; it was slow from 15-24°C. and very fast from 32-40°C. At the latter temperatures complete rotting occurred after 96 hours. There was some correlation between the thermal relation of the fungus in culture medium and on the fruits as evidenced by the rate of rotting.

5. The fungus tolerated a wide range of *p*H from 3·0 to 8·4, but thrived best at 4·2 to 5·0. The *p*H of green apple was found to be 4·2 and the ripe apple 5·2. Both these, therefore, afforded a suitable substratum for growth of the fungus.

6. The identity of the fungus is discussed and the species under study is assigned to *Rhizopus arrhizus* Fischer with the reservation as to its validity made by Zycha [1935] and as understood by him.

Acknowledgment

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A NOTE ON BOTRYTIS-ROT OF GRAPES IN THE QUETTA VALLEY

BY

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(With Plates XXXV—XXXVII and one text-figure)

Introduction

Botrytis-rot of grapes has been reported in Europe by Muller, Viala [1891], Ravaz, Cuboni and Berro Aguilera [1926], in America by Rose and Ballard [1927] and Perold [1927], and in South Africa by Deyer [1931], and Du Plessis [1934]. In India, it has been recorded for the first time by the author in the Quetta Valley. Two species of *Botrytis*, *viz.*, *Botrytis cinerea* Pers. and *Botrytis vulgaris* Fr., have been found responsible for the disease. The latter is reckoned by many workers as a sub-species of *Botrytis cinerea*. In Southern Europe, where *Sclerotinia fuckeliana* De Bary is found associated with *Botrytis cinerea*, and is reckoned to be the perfect stage of this fungus; the former name is in common use.

The disease is named gray rot, noble rot, porriture grise, porriture noble, granfaule, edelfaule, vaalvrot and *Botrytis*-rot, etc. The author prefers the name *Botrytis*-rot to gray rot as strictly speaking the colour of the mould is not gray but grayish olivaceous. The fungus conforms with *Botrytis vulgaris* Fr., described by Saccardo [1886]. No ascigerous stage has been observed in culture or in the fields associated with this fungus.

Description of the disease

Botrytis-rot of grapes is characterised by the decay and rot of berries associated with a grayish olivaceous growth of the fungus. The affected berries turn soft and brown and generate a pleasant smell. Infection usually starts from a cracked berry and spreads from it to other parts of the bunch. Sometimes the berries may crack before rotting. The fungus has been found to be capable of attacking healthy berries, though bruises and cracks facilitate its spread. Under humid conditions, the disease is fairly destructive and associated with powdery mildew of grapes takes a heavy toll of the crop.

Description of the fungus

Botrytis vulgaris Fr. Syst. Mycol. III, page 398, Sacc. F. Ital. T. 693, *Polyactis vulgaris* Link., Corda Ic. I. Fig. 250, *Botrytis polyactis* Link., *Monilia vulgaris*, Pers. Sacc. Syll. Fung. Vol. IV, Pp. 128-129.

Clusters grayish olive, hyphae in cottony masses, whitish olivaceous when young, becoming olivaceous with age, ascending to erect, septate, branching above; branches short, spready, secondary branches few, usually opposite; conidiophores large, erect, olivaceous, constricted, several millimeters high, 10-25 μ. thick, simple or branched; secondary branches 7-9 μ. thick near the head, gradually attenuated to 3-4 μ. at the ends. Heads of conidiophores sub-hyaline, granular, smooth or slightly verrucose. Conidia oval, oblong-oval or globose, 6-9 by 7-13 μ., 7 by 10 μ. predominant, granular, sub-hyaline appear grayish olivaceous in clusters, acrogenus or acropleurogenus.

Conidia germinate readily in distilled water and 0·1 per cent glucose solution at 30°C., one to three germ tubes having been observed (Plate XXXVI, fig. 3). Conidial formation is more abundant in light than in darkness. Infection, for the most part, takes place by the spread of these conidia. Germ tubes are capable of penetrating through healthy skin, the degree of penetration varying with the ripeness of the berries. Hyphae invade all parts of the berry, growing within the cells as well as between them, and ramifies through the tissue of grapes surrounding and filling them with mycelium. As a result of infection the cells are destroyed, the protoplasm shrinks away from the cell's wall and becomes disorganised.

Sclerotia.—Sclerotia are abundantly formed in culture (Plate XXXVI, fig. 1). In older growth certain hyphae twist round to form concentric coils of hyphæ which generally become thick to form minute knot-like bodies. These are the young sclerotia which are at first whitish in colour, but later develop a dirty greenish tinge. As they advance in age they turn dark brown and finally become almost black. Mature sclerotia (Plate XXXV, fig. 10-10c) are dark fuscus or black, 3-10 mm. in diameter, irregular in shape, hairy; hairs thick, black, coriaceous; wall of the sclerotium dark brown, of more or less polygonal cells, and under it is a hypodermal layer of dark brown cells and a central medullary zone of compact, thick, hyaline and granular hyphæ. Wefts of hyaline hyphæ connect the sclerotium with the mother mycelium and serve as feeders, when the sclerotia are young; the hairs seem to be mainly protective.

Secondary conidiophores.—After a period of rest which ranges from a few days to several weeks, the sclerotia form all over their surface conidiophores of the normal type (Plate XXXV, fig. 10-c) The vast majority of these conidiophores bear conidia in acropleurogenus manner. The conidia from these germinate and form new mycelia which in turn repeat the life-cycle. Conidial formation is more rapid in light than in darkness.

Microconidia.—Occasionally microconidia are formed. Certain conidia on germination form two germ-tubes, one of which develops into a haustorial filament, while the other forms a short fairly branched conidiophore which bears microconidia in chains (Plate XXXVI, fig. 2). These microconidia do not seem to possess any power of germination and are not of much consequence in the life-cycle of the fungus.

PLATE XXXV

FIG. 1. Conidiophore of *Botrytis vulgaris* with terminal cluster of acrogenus conidia, (× 100) 1 *a*. part of conidiophore with acropleurogenus arrangement of conidia (× 100)

FIG. 2. Head of conidiophore (× 625)

FIGS. 3—3*a* Showing different modes of branching of the head of the conidiophore (× 100)

FIGS. 4—6. Heads of certain types of conidiophores magnified (× 960)

FIG. 7. A secondary branch of the head of young conidiophore, bearing young conidia (× 960)

FIG. 8. Conidia (× 625)

FIG. 9. Germinating conidia with 1-3 germ tubes in different stages of development (× 625)

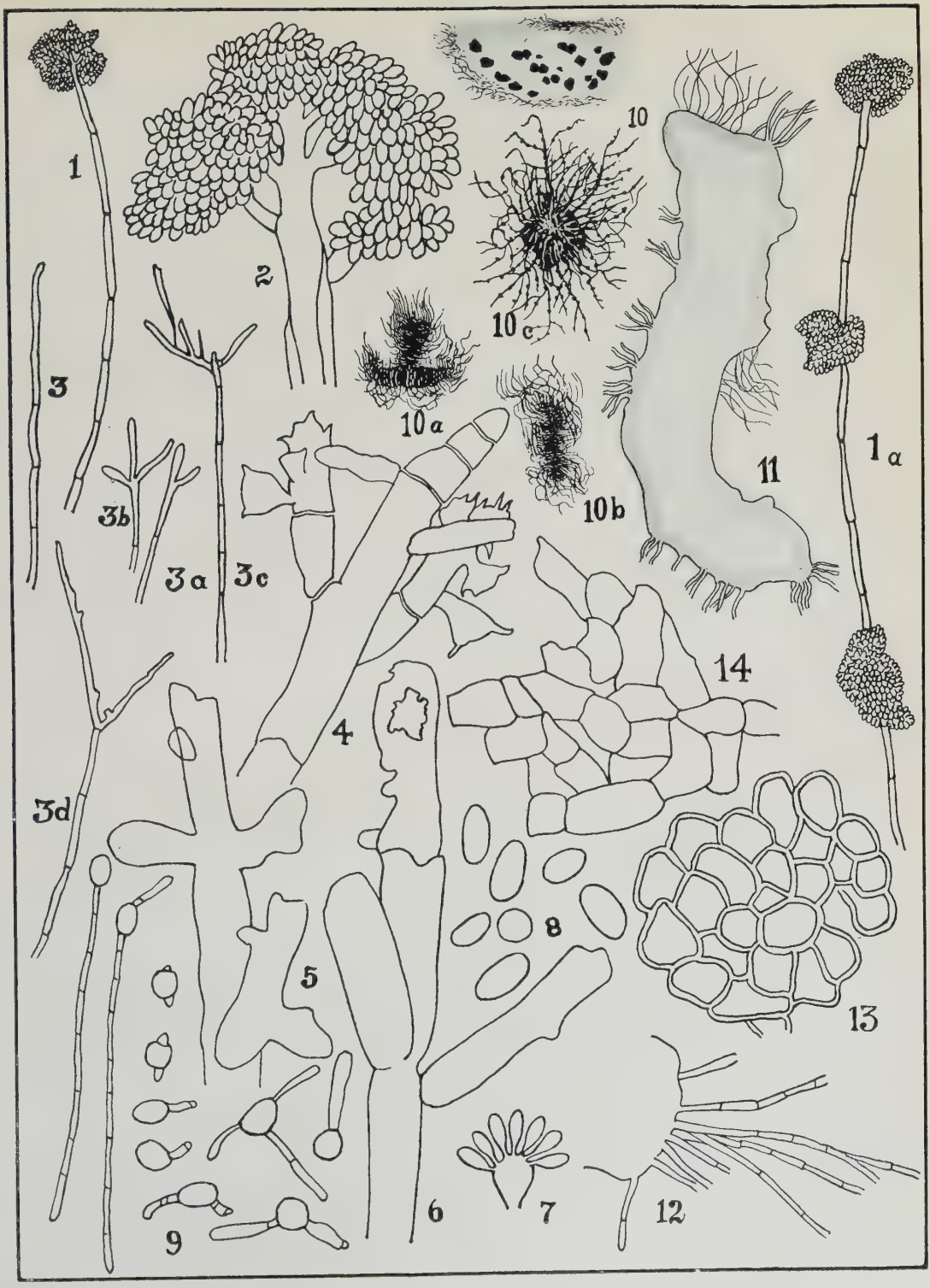
FIGS. 10–10*c* Sclerotia in different stages of development, 10*a* Young sclerotium in culture (nat. size), 10*a* 10*b* Mature sclerotia (×3), 10*c* Schlerotia with secondary conidiophores (×3)

FIG. 11. Section of the sclerotium showing sterile hairs coriaceous wall, hypodermal layer of dark brown cells shown light dark and central medullary zone of hyaline or sub-hyaline hyphae. These single threads shown as hairs represent sub-hyaline wefts of hyphae which are primary source of nutrient supply to sclerotium (× 100)

FIG. 12. Sterile hairs of sclerotium (× 100)

FIG. 13. Showing texture of the wall of the sclerotium (×960)

FIG. 14. Part of medullary zone of the sclerotium (×960)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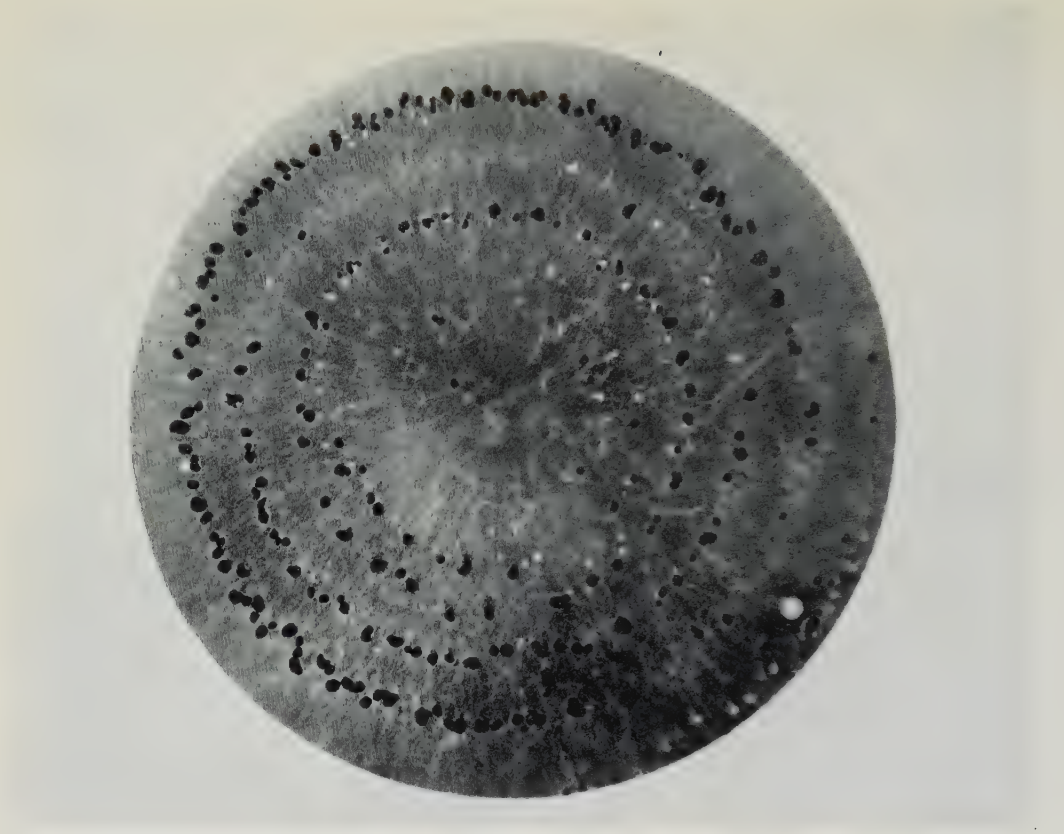

FIG. 1 Photograph of colony of *Botrytis vulgaris* Fr. on oat agar, incubated at 30°C. for 7 days, showing the formation of sclerotia in concentric rings

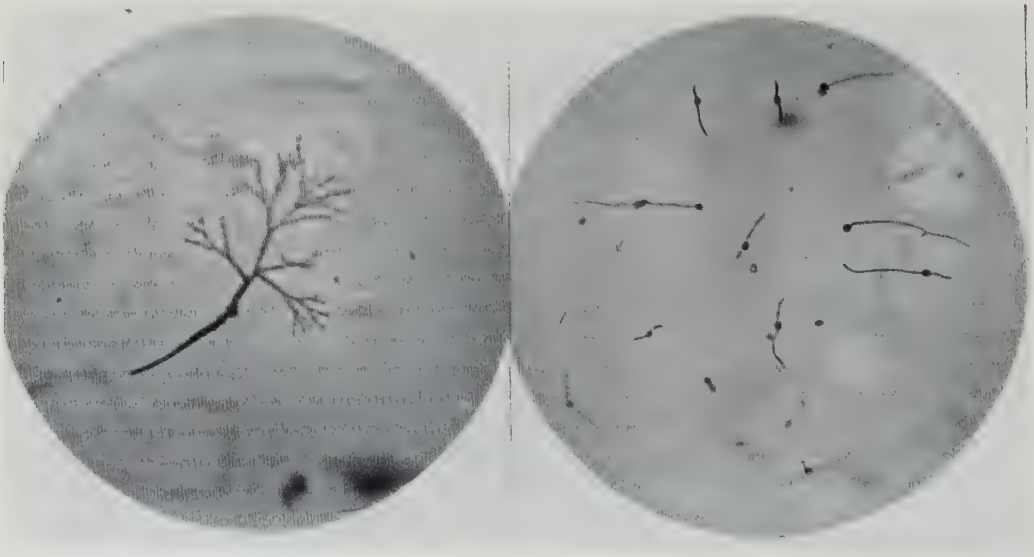

FIG. 2 Photomicrograph of a microconidiophore formed by the germination of a spore of *Botrytis vulgaris*. Microconidia are borne in chains

FIG. 3 Photomicrograph of germinating spores of *Botrytis vulgaris* ; 1 to 3 germ tubes are shown

FIG. 1 Photograph of a small bunch of grapes kept as control during inoculation experiments.

FIG. 2 Photograph of a small bunch of grapes artificially infected with spores of *Botrytis vulgaris* Fr. (after 5 days).

FIG. 3 Photograph of a small bunch of grapes artificially infected with spores of *Botrytis vulgaris* Fr. (after 10 days).

Anastomosis.—Anastomosis or hyphal fusions have been often observed between two germ-tubes or between hyphæ and germ-tubes, affording a possibility for the interchange of cell contents. This was first noted by De Bary and has been observed by many others. Recently Hansen and Smith [1932] have reported the occurrence of microconidia and anastomosis. The frequent occurrence of multinuclear condition in hyphal cells and spores of *Botrytis* and such other fungi has suggested to these workers the possibility of irregular distribution of genetically dissimilar nuclei in cell-division or conidial formation. This phenomenon has been the basis of considerable speculation in relation to the origin of variation in fungi.

Ascigerous stage.—No ascigerous stage has been found in culture or in the fields associated with this fungus. All attempts to stimulate sclerotia to form apothecia failed. The sclerotia were isolated from cultures and thoroughly washed with sterilised distilled water. These were then placed in dishes on moist sand, quartz and filter paper and kept at 7°C. in darkness for a period of two months, but no apothecial fundaments were observed; conidiophores, however, were formed in abundance. The sclerotia were kept under further observation for six months but no apothecia appeared.

Pathogenecity

Inoculation experiments were organised to study the pathogenecity of *Botrytis vulgaris* Fr. Small bunches of grapes were taken. The berries of four bunches were pricked with a sterilised needle. Two of these were washed with the suspension of *Botrytis* spores in sterilised distilled water and the other two were washed with uninfected sterilised distilled water; each bunch was kept in a pair of Koch's culture dishes containing small amount of sterilised distilled water to maintain humidity. Dishes were kept at the room temperature. Four more bunches of grapes were taken. All these were kept unbruised. Two of these were washed with suspension of *Botrytis* spores in sterilised water. Each bunch was kept in a pair of Koch's culture dishes as described above. Within 24 to 48 hours the infection took place in all the cases where the bunches were washed with suspension of spores, while the others remained unaffected. Within a week the infected grapes were badly infested with the fungus (Plate XXXVII, fig. 2) and showed signs of decay while the controls remained almost healthy. The infection in case of berries pricked with needles was very rapid as compared to those unbruised. The fungus is capable of piercing healthy cell-walls. The experiment was repeated and identical results were obtained.

Physiology of the fungus

Prune agar, standard synthetic agar, potato-dextrose-peptone agar, oat agar, Richard's media and Coon's agar were the media used for the cultivation of this fungus. The formulæ used in the preparation of these media were those recommended by Rawlins [1934]. Petri dishes of uniform size (9 cm. in diameter) were used and 20 cc. of media was poured in each dish to have a

uniform thickness. Six plates of each medium were poured and a small piece of mycelium bearing conidia was planted in the centre of each dish and plates were incubated at 30°C. The growth character and the spread of the fungus after incubation for one week is presented in Table I.

TABLE I

Growth character and spread of Botrytis vulgaris *Fr. in different media, at 30°C.*

Media	Growth character	Average spread of colony in 7 days (Diameter in mm.)
Prune agar . . .	Comparatively poor growth, sterile mycelia abundant, few or no sclerotia, sporulation poor	28·3
Standard synthetic agar .	No sclerotia, thin growth, conidiophores fairly common	26·7
Potato-dextrose-peptone agar	Thick, good flaky growth, vegetative growth predominant. Mycelia dirty white, no sclerotia, conidiophores comparatively poor	26·2
Oat agar	Sporulation, vegetative growth and sclerotial formation abundant	33·7
Coon's agar . . .	Sporulation, vegetative growth and sclerotial formation fairly good	27·1

The effect of different temperatures on the growth of *Botrytis vulgaris* was also studied. Oat agar was the medium used; 36 plates were poured ; each dish was of uniform size, containing 20 cc. of media. These were inoculated with the fungus and sets of six such plates were incubated at each temperature. Growth at six different temperatures, *viz.*, 7°C., 19°C., 23°C., 27°C., 30°C., and 37°C., was studied. Sporulation was most active at 7°C., it gradually diminished with advance in temperature. At 27°C., there was again intense sporulation ; then there was sudden fall and it came almost to a standstill at 37°C. Sclerotial formation was fairly active at 7°C. With the advance in temperature it increased and was at its highest at 27°C. Then it began to decrease and there was slight sclerotial formation at 37°C. As regards aerial mycelium, 23°C. was found to be the optimum temperature for its growth and at 37°C., mycelial growth was more or less completely inhibited. The effect of different temperatures on the growth of this fungus is shown in Table II and is graphically represented in Fig. 1.

TABLE II

Effect of different temperatures on the growth of Botrytis vulgaris *Fr.*

No.	Temperature	Aerial mycelium*	Sporulation*	Sclerotial formation*	Spread of colony in 7 days (diameter in mm.)
1	7°C.	+	++++	+++	38·8
2	19°C.	++++	+++	+++++	46·7
3	23°C.	+++++	++	+++++	78·5
4	27°C.	+++	++++	+++++++	27·3
5	30°C.	+	++	++++	26·6
6	37°C.	—	—	++	9·6

*—denotes absence or negligible presence.
+, ++ denote slight and moderate growth respectively.
+++ to +++++++ show varying density of occurrence.

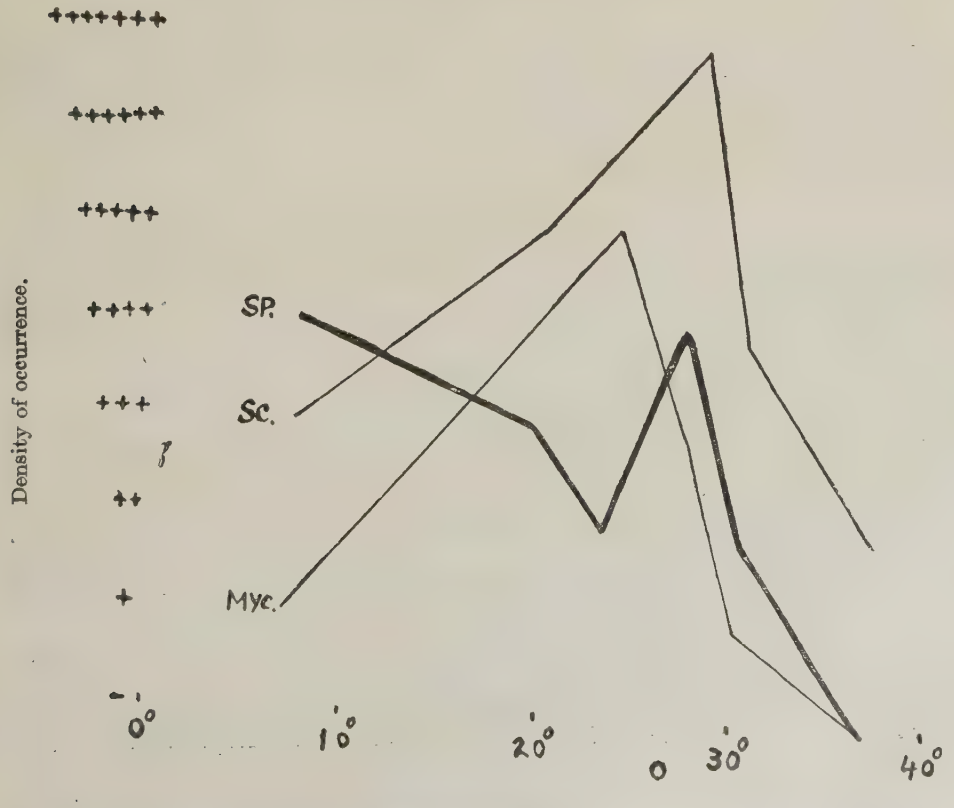

FIG. 1. Effect of various temperatures on growth and development of *Botrytis vulgaris*. Myc = spread of mycelium, Sc = sclerotial formation, and Sp = sporulation.

Summary

1. A rot of grapes due to *Botrytis vulgaris* Fr. is recorded.

2. A brief etiology of the disease is given.

3. Spores of *Botrytis vulgaris* germinate readily in distilled water at 30°C. and produce one to three germ-tubes.

4. Formation of microconidia and anastomosis between two germ-tubes or hyphæ and germ-tubes was observed.

5. No ascigerous stage was found associated with this fungus in culture or in the fields.

6. Oat agar and Coon's agar were found to be very suitable media for the growth of *Botrytis vulgaris*.

7. Correlation of temperature and growth of *Botrytis vulgaris* was studied. 27°C. was found to be the optimum temperature for the growth of mycelium and sclerotial formation, while sporulation was most active at 23°C. At 37°C. the growth of aerial mycelium of the fungus and formation of spores were inhibited but some sclerotia were formed.

Acknowledgments

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SOME NOTES ON EYE-STRIPES IN ACRIDIDAE*

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Attention has been drawn to the existence of stripes in the compound eyes of various species of Acrididae by systematists in their descriptions, though little significance seems to have been attached by them to this character. Roonwal [1936] was the first to notice in *Schistocerca gregaria* Forsk. (the desert locust), the existence of two types of individuals among the "solitaria" population found on the Mekran Coast—one possessing six stripes in the eyes, and the other, seven stripes. In his note, he observed: "The origin of this difference is, however, obscure. Whether this is inherited in a genetic way can only be shown by extensive breeding experiments." Subsequently, he studied the development of stripes in the hoppers of *Schistocerca* at Pasni, and came to the conclusion that, while in the six-striped type the number of eye-stripes increased at the rate of one for each moult, in the seven-striped one, two stripes were added in one of the hopper instars [Rao, 1938].

In the course of certain feeding experiments, however, Mukerji (working at Karachi) and Batra (stationed at Ambagh) had opportunities of following the development of a large number of hoppers, and their observations, made quite independently, showed that there was a perfect correlation between the number of moults passed through and the number of the stripes acquired by the hopper [Mukerji and Batra, 1938]. Thus in a five-instar life-cycle, an adult with six eye-stripes was produced, while a seven-striped eye was the result of the occurrence of an extra moult, or in other words, of a six-instar life-history. In a few cases, two extra moults were noticed, resulting in the production of adults with eight stripes in the eye. In a few other instances, Mukerji met with cases of a four-instar life-cycle accompanied by the production of a type with five eye-stripes.

Should these conclusions be confirmed, the eye-stripe character in *Schistocerca* would apparently serve as an easily recognisable index of the developmental history of the hopper stages of each individual, and may also serve

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to signify any possible tendencies in regard to change of phase. The senior writer had opportunities of examining a large series of specimens of *Schistocerca gregaria* gathered from actual swarms during past infestations in India, belonging to :—

(1) Karachi Locust Collections including *gregaria* forms collected in 1931 and a few specimens from Africa presented by Dr. B. P. Uvarov,

(2) Collections of the Imperial Entomologist, New Delhi (1904-1930),

(3) Collections of the Indian Museum, Calcutta (1889-1915), and

(4) Collections of the Professor of Entomology, Poona Agricultural College (1915-1930).

In all the *gregaria* forms subjected to scrutiny, he invariably found six stripes in the eyes (wherever the stripes were clearly visible), accompanied by 26 antennal segments in all such specimens as had the antennae intact. He also noticed that the specimens collected at Pasni and elsewhere during the time of the great incursion of locusts in July 1935, and the small incursion of May 1932 at Pasni [Rao, 1936] consisted predominantly of forms with six eye-stripes possessing *gregaria* ratios. In the course of biometrical work, the junior writer had ample opportunities of examining both *gregaria* and *solitaria* phase material of *Schistocerca* collected in the course of the last six years, from the various habitats of the desert locust within Indian limits. His observations go to show that collections of non-gregarious forms are generally made up of both six-striped and seven-striped types, though at certain times seven-striped ones were found predominating. The six-striped individuals, however, show mostly *solitaria* ratios, and usually possess 27 antennal segments, instead of the 26 found in *gregaria* forms. The seven-striped forms generally have 28 to 29 antennal segments, while eight-striped forms have 30 (as noted in a small number of specimens with eight eye-stripes found in nature in 1936). The dominance of the six-striped character in *gregaria* forms may, to a certain extent, be taken as an index of the rapidity of their developmental processes, resulting in the relative shortness of the hopper period as compared with that of the *solitaria* forms.

In certain other genera of Acrididae, in which eye-stripes are known to occur, a general correspondence of the number of stripes with the number of instars has been recorded in cases where the life-history has been worked out. In the case of *Patanga succincta* L. (the Bombay locust), eight eye-stripes have been noted to be quite common in the forms collected from swarms (1904-1910) ; and even among those met with in the desert in the course of locust surveys in recent years, eight-stripes are the general rule, though a few nine-striped forms have also been met with in the Rajputana desert. In addition, a few forms with seven eye-stripes have also been noticed in the collections. In this connection, it may be mentioned that Lefroy [1906] has recorded eleven cases of rearing of the Bombay locust from egg to adult,

of which nine hoppers passed through seven moults, while one underwent six and another eight moults. In the single specimen of *Nomadacris septemfasciata* Serv. (the Red-Locust of Africa), presented to the Karachi collection by the Imperial Institute of Entomology, London, seven eye-stripes are seen, which would correspond with the record of the general occurrence of six moults in its life-history [Faure, 1935]. In *Colemania sphenarioides* Bol. (the Deccan grasshopper), six to seven eye-stripes are found, which may be correlated with the occurrence of five or six instars recorded by Coleman [1911].

Six to seven vertical stripes are also to be found in the eyes of *Anacridium egyptium* L. (the Egyptian grasshopper), and in *Cyrtacanthacris tatarica* L. Similar prominent vertical eye-stripes are noticeable also in the following genera : *Caloptenopsis*, *Calliptamus*, *Thisoecetrus*, *Euprepocnemis*, *Dericorys*, *Spathosternum*, etc. Instead of straight and vertical stripes, curved, concentric ones are to be found in certain genera of the group *Pyrgomorphinae*, *viz.*, *Pyrgomorpha*, *Colemania*, and *Chrotogonus*. In *Acrida*, the stripes are parallel and horizontal (not vertical) in correspondence with the position of the eye in the anteriorly projecting snout of this form and may vary in number from seven to ten.

In certain Acrididae, stripes are conspicuous by their absence, the eye being generally irregularly marbled with cream and brown, as for example, in the genera, *Locusta* (*Locusta migratoria* L.), *Dociostaurus*, *Sphingonotus*, *Pyrgodera*, etc. In *Œdaleus*, however, stripes are visible in many specimens, though rather faintly.

In certain other genera, only two or three anterior stripes are clearly visible, the posterior ones becoming more or less masked by light brown, as, for instance, is the case with *Hieroglyphus*, *Catantops*, *Oxya*, and *Choroedocus*.

In *Hieroglyphus banian* F., an examination of a great number of live hoppers of all stages in the field in regard to the eye-stripes as well as the number of their antennal segments gave ample indications that, as in *Schistocerca*, the number of stripes progressively increases in this form with the number of moults passed through, it being noticed that the eye-stripes were generally clearer and more distinct in the hopper stages of *Hieroglyphus* than in the adult forms.

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DELINTING AND GERMINATION OF COTTON SEEDS AS AFFECTED BY $ZnCl_2.2HCl$ TREATMENT.

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(With four text-figures)

Preliminary to cotton cultivation, two problems confront its grower, *viz.*, (*i*) how to delint the seeds, and (*ii*) how to secure earlier and more regular germination. The dung treatment of fuzzy types, a common practice prevalent among the Indian peasants, though it helps a clear broadcast, does neither delint the seed nor affect the germination process. The presence of fuzz on seeds handicaps germination and also interferes in the isolation of dead and shrivelled seeds from the stock.

The importance of good germination is unique and in fact this phase is emphatically the most critical one in the plant's life-span. It is here that the seeds either perish because of limited moisture or rot due to excessive irrigation. A prompt and complete germination is, therefore, an essential preliminary to the future prosperity of plants, for it ensures, amongst other things, a regular full stand, a complete elimination or reduction of weeds, a protection against heat of the sun and a uniformity in their growth. To gain this end, treatments like scarification [Rose, 1915], alteration in temperature [Harrington, 1923 ; Morinaga, 1926], irradiation [Singh and Choudhri, 1938] and immersion in chemicals [Denny, 1926 ; Archibald, 1927] have been attempted by various investigators.

Unlike other crops, in cotton, a method that would supply the two essential needs detailed above would be of genuine economical worth and any step towards the achievement of this end would be an important addition to the success in the cultivation of cotton. Assuming that 90 per cent of cotton fibre consists of pure cellulose [Miller, 1931] which readily dissolves in $ZnCl_2.2HCl$ [Cohen, 1925 ; Miller, 1931], the present investigation was designed to test the validity of this method of seed treatment towards delinting and obtaining a more successful germination.

Method

Pure strains of two cotton varieties, *viz.*, C402 (Indian) and Perso-American, after careful grading for uniformity, were soaked in an acidic solution of zinc chloride (zinc chloride dissolved in twice its weight of concentrated hydrochloric acid) contained in a pneumatic trough for durations of 2, 5, 10, 15, 20, 30 and 60 minutes respectively. To ensure homogeneous action of the solution on seeds they were constantly stirred with the aid of a thick glass rod. At specified intervals lots of seeds were removed from the container with a glass spoon, and the acid solution drained off. The various lots were then transferred to sieves and subsequently washed under jets of running water for periods of not less than half an hour to remove completely the adhering acid. To provide basis for comparison of results, two types of checks were maintained (*i*) dry and (*ii*) soaked. The set of dry seeds was not subjected to any pre-sowing treatment while the seeds belonging to the other were soaked in water for half an hour to balance the effect of water washing in case of treated seeds. Since $ZnCl_2.2HCl$ solution contained no water, a water-soaked control for duration of more than half an hour was considered without use. The external moisture from both the treated and the soaked control was wiped off by putting the seeds in the folds of blotting papers. After another ten minutes the seeds were sown in big enamelled dishes under sand culture. The use of sand was preferred to ordinary soil in order to eliminate the effect of heterogeneity of the growing substratum, which would otherwise affect the experimental observations.

In all, ten sets of experiments were conducted with each of the two cotton varieties. In each set ten dishes were employed and in each dish 25 seeds were sown. The various lots were examined every morning and evening and germination counts made which for a tacit appreciation of the data were computed per hundred and analysed statistically using each dish as a replication.

Results

Delinting.—Cotton fibre furnishes the best example of almost pure normal cellulose, hygroscopic in character and insoluble in ordinary solvents. Concentrated sulphuric acid is said to dissolve it but gradually [Morinaga, 1926]. The dissolution of fuzz was secured more readily on treatment of seeds with $ZnCl_2.2HCl$ solution. It depended, however, to a great extent upon the variety of cotton investigated. In type C402, for instance, about three-fourths of the fuzz dissolved only after two minutes' soaking, while complete delinting occurred after five minutes' duration. Perso-American belonging to *hirsutum*, which is noted for its larger surface and greater fuzz than the *desi*, required obviously a longer treatment of ten to fifteen minutes' duration for the entire removal of fuzz from seeds. In this case treatment up to five minutes was found to be insufficient to bring about this change. Approximately one-fourth of the fuzz after two minutes' immersion of seeds and about half or

slightly more after five minutes dissolved in $ZnCl_2.2HCl$ solution, which indicated the differential response of the two varieties, possibly due to differences in the strength of fuzz and floss.

Germination effects.—In order to know the comparative details of seed germination of the two types of cotton, the counts made every morning and evening on both the controls and the treated samples are presented in detail in Table I.

TABLE I

Comparative changes in germination of control and treated cotton seeds belonging to types C402 and Perso-American

Treatment	No. of seeds	Number germinated after an interval of (days)								Total	Germination capacity (percentage)	Duration of germination (days)
		½	1	1½	2	2½	3	3½	4			
					C 402							
Control (dry) . .	250	0	100	53	20	10	10	0	0	193	77·2	3
Control (soaked) . .	,,	0	27	28	37	5	3	0	0	100	40·0	3
$ZnCl_2 \cdot 2HCl$ 2 mins. .	,,	80	125	35	3	0	0	0	0	243	97·2	2
,, 5 mins. .	,,	72	128	45	0	0	0	0	0	245	98·0	1½
,, 10 mins. .	,,	78	133	32	0	0	0	0	0	243	97·2	1½
,, 15 mins. .	,,	17	188	15	10	0	0	0	0	230	92·0	2
,, 20 mins. .	,,	0	193	23	0	2	0	0	0	218	87·2	2½
,, 30 mins. .	,,	0	132	33	17	13	0	0	0	195	78·0	2½
,, 60 mins. .	,,	0	125	33	20	10	0	0	0	188	75·2	2½
					Perso-American							
Control (dry) . .	250	0	0	18	5	15	15	7	0	60	24·0	3½
Control (soaked) . .	,,	0	38	58	33	7	12	5	0	153	61·2	3½
$ZnCl_2 \cdot 2HCl$ 2 mins. .	,,	12	38	53	35	5	10	2	0	155	62·0	3½
,, 5 mins. .	,,	12	43	65	38	5	7	0	0	170	68·0	3
,, 10 mins. .	,,	15	67	43	45	8	17	0	0	195	78·0	3
,, 15 mins. .	,,	0	52	53	15	30	23	0	0	173	69·2	3
,, 20 mins. .	,,	3	42	58	12	17	13	5	0	150	60·0	3½
,, 30 mins. .	,,	0	43	42	20	22	5	3	0	135	54·0	3½
,, 60 mins. .	,,	0	35	48	12	10	13	0	0	118	47·2	3

It is well indicated that external emergence of the embryo did not take place simultaneously in all the sets and naturally, therefore, the percentage germination was to be affected. Even in those cases where sprouting began at the same time and on the same day, this aspect greatly differed from set to set. It was experienced that lots, where germination startedearlier or where

a good many seeds germinated at the commencement of the process, usually gave good account of themselves at the end. With a view to determining then the degree of reliance on such variations arising out of treatments, the essential data are subjected to critical statistical test and analysis of variance worked out as shown in Table II.

TABLE II

Analysis of variance of germination percentage

Due to	Degrees of freedom	Sum of squares	Mean square	Ratio of variances
C402				
Blocks . .	9	637·1	70·78	..
Treatments . .	8	26969·6	3371·20	83·27
Error . . .	72	2914·9	40·48	..
Total .	89	30521·6	..	..
Perso-American				
Blocks . .	9	730·0	81·11	..
Treatments . .	8	19440·3	2430·00	78·38
Error . . .	72	2235·1	31·02	.
Total .	89	22405·4	..	..

According to the above scheme, the ratio of variance due to treatments in both types of cotton is significant even at 1 per cent level. This would indicate that the differences noticed in seed germination were not mere chance variations but were the actual after-effects of seed treatment with $ZnCl_2.2HCl$.

Out of the many cases thus experimented, those exhibiting the most pronounced variations from day to day in the two types of cotton have been plotted as daily total percentages (Figs. 1 and 2) to indicate more clearly their departure from the normal.

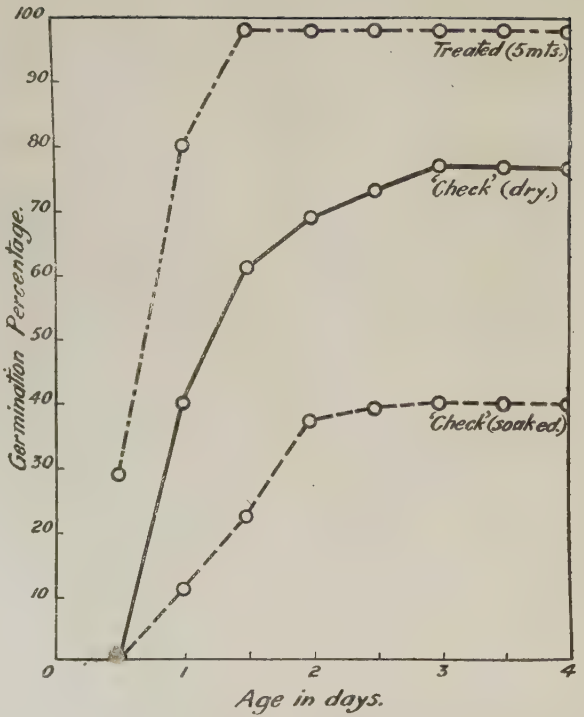

FIG. 1. Total germination percentages of control and treated cotton seeds (*Var.* C402) on days following sowing

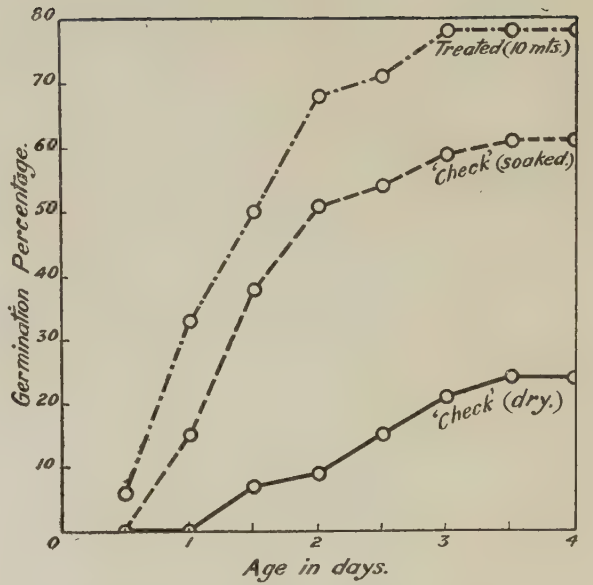

FIG. 2. Total germination percentages of control and treated cotton seeds (*Var.* Perso-American) on days following sowing

Whereas it is evident that the treated lot began to germinate earlier and had a higher initial start, it is also noticed that the total percentage figures in the treated set by subsequent additions maintained consistently a level higher than the control. This is true for the majority of the doses tried in this investigation though the degree of change was far too less in some cases than in others (Table I). Some heavier doses, for instance, those of one hour duration, did not yield promising data in this regard.

A careful examination of Figs. 1 and 2 further showed that soaking of seeds in water above had a profound influence upon their capacity to germinate, but this effect on the two types of cotton was dissimilar. The American variety showed better germination than its dry control, while C 402 was adversely affected. The treated samples deviated so significantly from both types of checks that the ultimate effects could not be attributed to water treatment.

For evaluating the relative significance of germination data obtained with regard to different treatments, critical difference has been computed in each of the two cases and percentage counts arranged in descending order as shown in Table III.

TABLE III

Treatments arranged in order of effects produced

C402

Critical difference=7·49

Treatments (mins.)	5	2	10	15	20	30	Control (dry)	60	Control (soaked)
Germination percentage	98·0	97·2	97·2	92·0	87·2	78·0	77·2	75·2	40·0

Perso-American

Critical difference=6·34

Treatments (mins.)	10	15	5	2	Control (soaked)	20	30	60	Control (dry)
Germination percentage	78·0	69·2	68·0	62·0	61·2	60·0	54·0	47·2	24·0

From the above table it is clear that most of the treatments produce significant effects with respect to dry control, while those ranging from two to fifteen minutes' duration are specially noted for their high level of significance.

Another fact of importance that could not escape notice was that the curves exhibiting total germination percentage for the treated lots (Figs. 1 and 2) were characterised by greater steepness in the earlier than the later stages.

This fact was more clearly exhibited by the curves for Indian cotton than those for the American, where the germination was comparatively gradual. A perusal of Table I would further strengthen the point. In the case of C402 over 50 per cent of germination occurred in every one of the treated sets within the first 24 hours of sowing inasmuch as some lots exhibited even 80-84 per cent. Thus, lots showing very high germination on the first day completed the same within another twelve hours' time after which germination was constant in these sets. On this account such curves were almost abrupt against the more smooth ones of the control where the percentage germination was gradual though less, and covered a much longer duration.

Considering the data collectively in several useful directions, *e.g.*, speed, duration and capacity of germination (Table I), it is noted that the suitable doses with regard to type C402 were those of two to ten minutes' duration, that of five minutes yielding the best results. Such doses hastened germination, took a much shorter time to complete it, and initiated a very high percentage of seed to sprout as compared with "checks". The same was true for American cotton as well, where treatments for five to fifteen minutes were more satisfactory than others while the one applied for ten minutes induced the best results. Below and beyond the best dosages there was a gradual diminution in the percentage capacity of germination of seeds which sprouted comparatively late and took a longer period to finish it. The great efficacy of the treatment of cotton seeds with $ZnCl_2.2HCl$ was, however, evidenced by the readiness with which this solution acted even after two minutes' application.

In American cotton, the percentage of germination was usually greatly enhanced, even as much as 54 per cent as compared to its dry control while the duration was not much affected. In Indian cotton, on the contrary, though the percentage increase was only 20-21, the speed of development was high and the duration of germination almost halved. In fact the latter type is more acclimatised to the conditions of this country and hence as many as 77 per cent of the seeds germinated even without any treatment, with much less scope left for such treatments. Smaller increase in the percentage germination of type C402 following $ZnCl_2.2HCl$ immersion, therefore, should not be attributed to the inefficiency of this solution. Rather, from many other view-points (*cf.* heights and dry matter accumulations) it appeared that type C402 was more advantageously affected by this pre-sowing treatment than Perso-American.

Addendum

While the object of the present work was solely to determine the effectiveness of this method of seed treatment towards germination, which phase in itself is the most important and critical one, measurements of the seedling growth were also made to know whether the effects produced, if any, were transitory or permanent. A large number of plants from each of the lots (which rendered fortuitous fit of observation very unlikely) were examined with regard to their heights, accumulation of dry matter and other essential details. The average data obtained from these measurements have been represented graphically in Figs. 3 and 4.

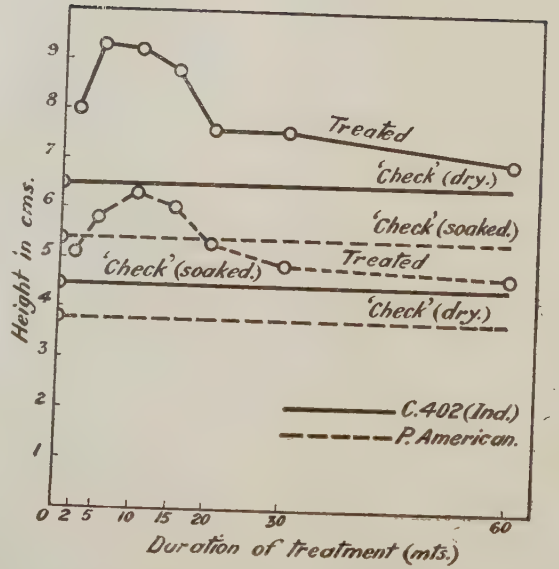

FIG. 3. Average heights of one week old cotton seedlings, shown to express the results on a comparative basis with regard to the duration of treatment provided. The 'checks' are indicated by straight lines

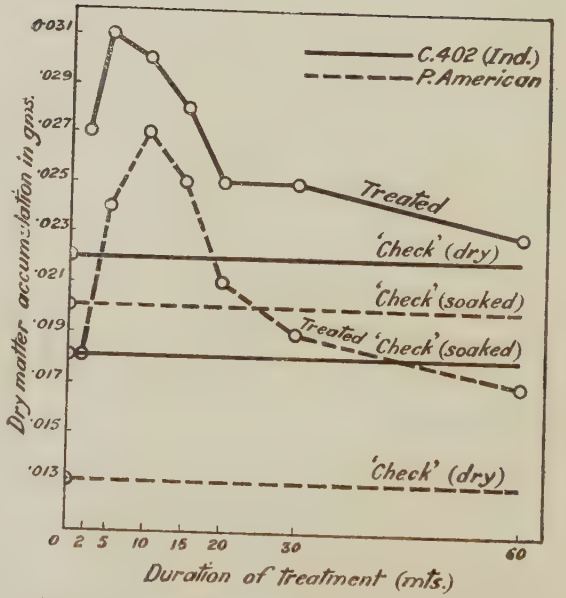

FIG. 4. Dry matter accumulation of one week old average cotton seedlings, shown to express the results on a comparative basis with regard to the treatment provided. The 'checks' are indicated by straight lines

It was observed that a number of control seedlings showed poor development, drooping habit and scant growth. Also, a fair percentage of mortality amongst them was evident after some time with little or no protrusion of the plumule. In treated sets, particularly those the seeds of which were subjected to five, ten or fifteen minutes' immersion, plants were invariably healthy, erect, firm and had a better stand. Such plants were also taller (Fig. 3) and accumulated greater dry matter (Fig. 4) than their respective controls. Of the types of cotton thus investigated, treated seedlings of C402 were radically different from their controls. On the whole, the general trend of these observations was very much like those noted on germination.

Summary

The data recorded in this paper were gathered to estimate the degree of delinting and germination effects in Indian and American varieties of cotton following seed immersion for various durations in $ZnCl_2.2HCl$. The experiments were conducted in sand culture and the enquiry which was proposed in the first instance to be restricted to the germination phase alone was later extended to the determination of seedling growth as well.

The solution used in this work readily dissolved the fuzz present on cotton seeds. The seeds of American type were found to be slightly more resistive where for complete isolation of the fuzz, 10 to 15 minutes' soaking was essential against five minutes in the case of type C402. Doses shorter than five minutes were insufficient to cause complete delinting.

The germination process was greatly activated by $ZnCl_2.2HCl$ treatment of seeds. Doses of five to fifteen minutes' duration were very satisfactory in this regard. Such treatments initiated early start, higher percentage and early completion of germination. Shorter or longer treatments induced gradual depletion in the germinating vigour of seeds from the optimal value, though in the majority of cases germination was still superior to the controls.

The permanency of the effects of these treatments was well evidenced by the resulting seedlings which were taller and accumulated greater dry matter than their controls.

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THE HOT FERMENTATION PROCESS FOR COMPOSTING TOWN REFUSE AND OTHER WASTE MATERIAL*

I. INTRODUCTORY

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SINCE Hutchinson and Richards [1921] first worked out the scientific principles underlying the conversion of straw and other organic matter into synthetic farmyard manure, the question of composting waste materials—agricultural or town wastes—has been attracting a good deal of attention both in this country and abroad. Based on the essential principles of Hutchinson and Richards, but involving differences in technique to suit local climatic and labour conditions, the nature of the raw material, etc., various processes have been standardised for the preparation of compost—*e.g.*, the " Adco " process of Richards [1921], the Indore process of Howard and Wad [1931], the " activated compost " process of Fowler [1934], etc. Common features of the above methods are :

(a) that they aim at a rapid and efficient disintegration of the original material by the addition of a suitable nitrogenous starter so as to promote rapid microbial development ;

(*b*) that the decomposition is essentially aerobic and, generally, the compost is turned over a number of times to promote aeration ; in some cases, even underground flues are provided to increase air supply to the inner portions of the heap [Eden, 1938] ; and

(*c*) that the final aim is to obtain a well decomposed material, friable and easily broken down to a finely divided condition, generally brown to black in colour and with a C : N ratio near 10 :1.

Few systematic studies have been reported of the carbon and nitrogen transformations taking place in heaps composted under large-scale conditions of farm or town practice, and of the ultimate gains or losses of nitrogen occurring during such composting process. The accuracy of the data has, in general, been vitiated by the heterogeneous nature of the waste material—be it farm or

*The investigations reported in this and the following papers of this series were carried out at the Indian Institute of Science, Bangalore under a scheme ' for the preparation of cheap synthetic manure from town refuse and other waste material ' financed by the Imperial Council of Agricultural Research.

town waste—and the difficulty of sampling involved while dealing with cart-loads of such heterogeneous material. The data, however, that have so far been presented [Ayyar, 1928, 1933] ; [Jackson and Wad, 1934] ; [Jackson, Wad and Panse, 1934] ; [Rao and Subrahmanyan, 1935] ; [Rao, 1936] have shown that in the ordinary methods of aerobic composting adopted in this country and elsewhere, heavy losses of carbon and of nitrogen occur to the extent of 40 to 60 per cent of the amounts originally taken.

The loss of nitrogen is particularly serious in the composting of town wastes, *e.g.*, street sweepings and night soil, the latter of which possesses a C : N ratio near 8 : 1. It is difficult to widen the C : N ratio of such a compost heap to the desired level of about 30 : 1 at the start, since the street sweepings in India contain a good proportion of leaves which are also rich in nitrogen. Under these conditions, the loss may easily amount to over 50 per cent of the nitrogen originally taken. Rao and Subrahmanyan [1935] found that when town-wastes were sprayed with emulsified or previously liquefied night soil over a number of days, the nitrogen content rose up to 1·2 per cent in the case of composts treated with night soil emulsion and to 0·7 per cent. when liquefied night soil was used. On making further additions of nitrogen on subsequent days, the nitrogen content remained stationary at the above levels, indicating a complete loss of all the added nitrogen. Losses of nitrogen from 20 to 40 per cent are reported by Ayyar [1928, 1933] and Rao [1936] even while using inorganic nitrogenous starters such as calcium cyanamide, ammonium sulphate or sodium nitrate for composting purposes, in place of night soil.

Apparently, the aerobic conditions of decomposition adopted in the above cases favoured excessive losses of nitrogen and of organic matter, especially when the mass was turned over at intervals to promote humification. A modification, wherein the access of air could be controlled to a greater extent, but at the same time the humification of the material may be allowed to proceed, offered hopes of a better conservation of nitrogen and of organic matter.

A method which promised to yield satisfactory results in respect of the above requirements was the hot fermentation method, first brought to public notice by Krantz [1925], who successfully incorporated it in his well-known Edelmist process for the preparation of farmyard manure.

Krantz recommended special equipment and a definite arrangement of packing the material and devised his method originally for the efficient storage and ripening of farmyard manure. Various workers [Ruschmann, 1927 ; Boetskaul, 1927 ; Fruwith 1928 ; *vide* also Cunningham, 1927 ; Jenkins, 1935] have since tried the method for the production of farmyard manure from dung and stall litter and have claimed a higher conservation of nitrogen and of dry matter and a higher crop response for the method than is obtainable by the aerobic methods usually adopted.

The essential principle underlying the hot fermentation process is that the material is allowed to decompose aerobically, under optimum conditions of moisture and nitrogen supply, for a limited period (about five or six days)

during which time rapid decomposition takes place and a high temperature (60° to 70°C) is reached. When the temperature begins to fall, the supply of air is cut off by compacting the mass and pressing it down with a layer of fresh material or by soil or otherwise.

Preliminary trials carried out by Shrikhande *et al.* [1936] at Bangalore showed that the hot fermentation method was peculiarly suited to the composting of town wastes with inorganic nitrogenous starters such as ammonium sulphate, calcium cyanamide, sodium nitrate or organic starters such as night soil. The data obtained (*vide* Table I) showed that there was complete recovery of nitrogen by the method, except in the case of calcium cyanamide, when the initial C : N ratio was 30 : 1 ; but there was increasing loss at narrower ratios, especially at 10 : 1. The losses in dry matter, in the hot fermentation method were of the order of 15 to 20 per cent. The high temperatures attained during the process served to destroy fly-larvae and pathogenic organisms, and the final product obtained, even when night soil was the base, possessed no bad odour.

TABLE I

Composting of town refuse

Form of nitrogen added to refuse	C : N ratio at the commencement											
	30 : 1				20 : 1				10 : 1			
	Dry matter recorded	Total nitrogen before composting	Total nitrogen after composting	Per cent nitrogen in the finished product	Dry matter recorded	Total nitrogen before composting	Total nitrogen after composting	Percent nitrogen in the finished product	Dry matter recorded	Total nitrogen before composting	Total nitrogen after composting	Per cent nitrogen in the finished product
	lb.	lb. oz.	lb. oz.		lb.	lb. oz.	lb. oz.		lb.	lb. oz.	lb. oz.	
Ammonium sulphate . .	216	3 12	3 13	1·79	209	5 12	5 3	2·49	205	8 12	7 8	3·62
Sodium nitrate . . .	216	3 12	3 13	1·82	237	5 12	4 14	2·13	240	8 12	6 3	2·74
Calcium cyanamide . .	207	3 12	2 9	1·19	228	5 12	5 0	2·06	224	8 12	6 0	2·45
Night soil	224	4 9	4 8	1·47	...	...	...	...	...	...	...	...

Each cistern contained originally 250 lb. of refuse

In view of the encouraging results obtained in the preliminary trials, it was deemed advisable to examine the method more critically ; to study the various factors influencing the efficacy of the process and to standardise the conditions for securing optimum decomposition ; to compare the hot fermented

compost with that obtained by aerobic methods in regard to manurial efficiency, by pot and plot trials as well as by laboratory analysis and nitrification tests. The results of such investigations are presented in the succeeding papers of this series.

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ESTIMATION OF POTASH IN SOILS BY THE COBALTINITRITE METHOD

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In this paper an attempt has been made to test the efficiency of the cobaltinitrite method—volumetric and gravimetric—for the estimation of potash in pure salts and in hydrochloric and citric acid extracts of soils. In computing the results, the formula, $K_2NaCo(NO_2)_6.H_2O$ of Addie and Wood [1900] has been employed. The results have been frequently checked with those obtained by the standard platinichloride (Lindo-Gladding) and perchlorate methods.

The method of Addie and Wood [1900] has been tested and modified previously by several workers, chiefly by Drushel [1907, 1908], Bowser [1909], Dennet [1929], Milne [1929], Van Rysselberg [1931], Hibbard and Stout [1933], Lewis and Marmoy [1933] besides others; but due to variations in the technique adopted by most of them, especially with regard to the method of precipitation, the preparation of the reagent, the concentration of the solution at the time of adding the reagent, the length of time allowed before the filtration of the precipitate, the nature and reaction of the potassium permanganate and the final treatment of the precipitate which differed in composition under certain conditions, the validity of the theoretical factor for potash based on Addie and Wood's formula was doubted. An elaborate study of the several methods indicated that with more constant conditions of precipitation and titration of the resulting compound with neutral, instead of acid—potassium permanganate—as in the case of Drushel's procedure, concordant results could be obtained by adopting Addie and Wood's formula. It was also found that the cobaltinitrite method gave as consistent results as the other accepted ones and was at the same time considerably quicker. Removal of basic and acid radicals such as calcium, magnesium, sulphates, phosphates, etc. was found to be unnecessary.

The preparation of the reagent and the details of the various experiments conducted, with the results obtained under each, are set forth below.

Experimental

Preparation and properties of the cobaltinitrite reagent

The reagent was prepared according to the method of De-Koninck [1899] as detailed below :—

Thirty grams of cobaltinitrate are dissolved in 60 c. c. of water and mixed with 100 c. c. of saturated sodium nitrite solution (about 50 grms. in 100 c. c.

water). The mixture is then treated with 10 c. c. of glacial acetic acid and warmed for a while over a steam oven or water bath. The nitrous fumes evolved are then evacuated by means of a filter pump and the whole allowed to settle for 24 hours. The reagent is then filtered and kept in the dark in a coloured bottle. It is more sensitive than platinum chloride : a drop of 0·1 per cent potassium chloride solution in 5 c. c. of water will give the reaction for potassium immediately the reagent is added. The reagent is quite stable for a fortnight but after this period it is not reliable.

Experiment 1

Estimation of potash in pure salts.—As a preliminary to the final application of the method to soils and artificial mixtures of potash, it was tested with pure analytical reagents with special reference to the influence of common acid radicals on potash estimations. The technique adopted for the volumetric estimation was essentially that of Drushel [1907, 1908] which in brief is as below :—

The solution containing potash is treated with an excess of cobaltinitrite reagent prepared according to De-Koninck [1899] and the whole mixture evaporated to a pasty consistency in acetic acid medium on a water bath. The precipitate, after washing with 10 per cent acetic acid, is oxidised directly with standard $N/10$ or $N/20$ potassium permanganate and the potash estimated by adopting the factor 1 c. c. $N/10$ potassium permanganate = 0·0008564 grm. of potassium oxide.

For the gravimetric estimation, the precipitate is dried to constant weight in an air oven at 120°C. and the potash estimated by applying the conversion factor 0·2074 based on Addie and Wood's formula namely, $K_2NaCo(NO_2)_6 .H_2O$.

The results obtained for the various salts including potassium sulphate by the cobaltinitrite, gravimetric and volumetric procedures, as well as by the official platinichloride method (Lindo-Gladding) are given in Tables I and II, along with the percentage recovery of potash (K_2O) under each, taking the theoretical value as the standard.

TABLE I

Recovery of potash from pure salts by the platinichloride and Cobaltinitrite (volumetric and gravimetric) methods

Salts used for analysis	K_2O theoretical value mg.	K_2O by the cobaltinitrite method. Average of four determinations		K_2O by the platinichloride method. Average of four determinations mg.	K_2O recovered by the Cobaltinitrite method		K_2O recovered by the platinichloride method per cent
		Gravimetric mgms.	Volumetric mgms.		Gravimetric per cent	Volumetric per cent	
1. Potassium chloride A. R. (KCl) . .	53·2	52·8	53·0	52·9	99·25	99·62	99·43
2. Potassium phosphate A. R. (KH_2PO_4) .	31·2	31·1	31·1	31·0	99·68	99·68	99·36
3. Potassium nitrate A. R. (KNO_3) .	38·0	37·7	37·8	37·8	99·21	99·47	99·47
4. Potassium sulphate A. R. (K_2SO) .	44·1	43·9	44·0	43·8	99·55	99·75	99·32

TABLE II

Influence of common acid radicals on the volumetric determination of K_2O by the Cobaltinitrite method

Salts used for analysis	K_2O theoretical value mg.	K_2O estimated, average of four determinations mg.	Deviation of the estimated value from the theoretical mg. + or —	K_2O recovered per cent
1. Potassium chloride A. R. (KCl) .	53·20	52·90	—0·30	99·44
" " " " .	5·32	5·30	—0·02	99·62
2. Potassium phosphate A. R. (KH_2PO_4)	31·20	31·00	—0·20	99·36
" " " "	3·12	3·14	0·02	100·61
3. Potassium nitrate A. R. (KNO_3) .	38·00	37·80	—0·20	99·47
" " " " .	3·80	3·77	—0·03	99·21
4. Potassium sulphate A. R. (K_2SO_4) .	44·10	43·90	—0·20	99·55
" " " " .	4·41	4·38	—0·03	99·32

The figures show that the recovery of potash by the cobaltinitrite methods —volumetric and gravimetric—is not only quantitative but also agrees very closely with that of the standard platinum chloride procedure. To test the accuracy of the formula of Addie and Wood adopted during the course of this investigation the following indirect experiment was conducted.

Experiment 2

Ten c. c. aliquots of pure potassium sulphate (K_2SO_4 A. R.) solution equivalent to 0·043 grm. potassium oxide were treated with 10 c. c. of cobaltinitrite reagent and the ratio of K : Na in the medium varied from 1 : 30 to 1 : 200 by adding pure sodium chloride solution. The precipitates obtained were dried at 120°C. to constant weights and the potassium oxide calculated from Addie and Wood's formula. The dried precipitates were then dissolved in hot dilute hydrochloric acid and the potash estimated in the resulting solutions by the perchlorate method. The results obtained by both the methods are recorded in Table III. It may be seen that the results agree very closely with one another, thereby confirming the correctness of the formula, in spite of the wide variations in the ratio of K : Na from 1 : 30 to 1 : 200.

TABLE III

Gravimetric estimation of K_2O by the cobaltinitrite and perchlorate methods

Amount of K_2O in the medium (theoretical value) (Mg.)	Ratio of K : Na	K_2O recovered by		Per cent error in K_2O recovery by	
		Cobaltinitrite method (average of four estimations) (Mg.)	Perchlorate method (average of four estimations) (Mg.)	Cobaltinitrite method + or—	Perchlorate method + or —
K_2SO_4—10 c. c. equivalent to 43·2 mg. K_2O . .	1 : 30	43·3	43·1	+0·23	—0·23
Ditto . .	1 : 40	43·0	43·4	—0·46	+0·46
Ditto . .	1 : 50	43·1	43·3	—0·23	+0·23
Ditto . .	1 : 60	42·9	43·0	—0·70	—0·46
Ditto . .	1 : 75	43·3	43·2	+0·23	*Nil*
Ditto . .	1 : 100	43·0	43·3	—0·46	+0·23
Ditto . .	1 : 200	42·9	43·1	—0·70	—0·23

Experiment 3

This was carried out to study the conditions affecting precipitation of potash, and the subsequent treatment of the precipitate. The results obtained under different conditions are recorded in Table IV.

TABLE IV

Potash estimation by the cobaltinitrite (volumetric) method under different conditions of precipitation and treatment of the precipitate

Nature of precipitation and treatment of the precipitate	K_2O (theoretical) (Mg.)	K_2O recovered (average of four determinations) (Mg.)	Deviation of the estimated value from the theoretical (Mg.)	Percentage error of K_2O recovered + or —
1. Solution containing potash evaporated to a pasty consistency, the precipitate treated with $KMnO_4$ acidified with 1 : 1 H_2SO_4 (10 c. c. for every 200—300 c. c. of $KMnO_4$ as finally diluted) . . .	21·6	23·2	1·6	+7·40
2. Evaporated the potash solution to a pasty consistency, treated the precipitate with cold $KMnO_4$, raised the whole to a boil and then added 10 c. c. of 1 : 1 H_2SO_4 as in 1. .	21·6	21·8	0·2	+0·92
3. Solution containing potash concentrated to 15—20 c. c.; Cobaltinitrite reagent added to the cold solution and the mixture allowed to stand for 24 hours; the precipitate then treated with neutral $KMnO_4$ at the boiling point, followed by the addition of 10 c. c. of 1 : 1 H_2SO_4 as in 1 and 2	21·6	21·9	0·3	+1·39
4. Potash solution evaporated to a pasty consistency; precipitate added to neutral $KMnO_4$ at the boiling point and subsequently to H_2SO_4 (1 : 1) as in 1 and 2 . .	21·6	21·4	0·2	—0·92

It is evident from the table that the treatment of the precipitate with potassium permanganate acidified previously affects the results considerably, the percentage error being as high as 7, if the usual factor, 0·0008564 gram potassium oxide is used for every c. c. of a deci-normal potassium permanganate. This difference in value may be due to the oxidation of the precipitate with acid permanganate instead of neutral, thereby affecting the potassium equivalent of K_2O. In such cases, a new empirical factor has to be worked out to get at the real value of potassium oxide as has been done by Milne [1929].

Experiment 4

Influence of common bases on potash estimation.—The individual influence of iron, alumina and magnesium added as sulphates and calcium in the form of carbonate on potash values was studied by keeping the potash constant and varying the quantities of bases. The results presented in Table V go to prove the reliability of the method even under different conditions. The mixtures were prepared to conform to the minimum and maximum quantities of bases that might be present in 50 c. c. of hydrochloric acid extract of 20 grms. of ignited soil made up to 500 c. c. The percentages of iron and alumina have been varied from 10 to 50 each and lime and that of magnesia from 0·1 to 10.

TABLE V

Influence of bases on the volumetric estimation of K_2O by the cobaltinitrite method

Metalic salts used	K_2O added (theoretical) (Mg.)	K_2O recovered (average of four determinations) (Mg.)	Deviation of the K_2O recovered from the theoretical value (Mg.) + or —	Per cent. error of K_2O recovered + or —
I				
Ferrous sulphate ($FeSO_4.7H_2O$) equivalent to—				
1. 0·25 gm. Fe_2O_3	21·60	21·76	+0·16	+0·74
2. 0·5 gm. Fe_2O_3	21·60	21·78	+0·18	+0·83
3. 0·75 gm. Fe_2O_3	21·60	21·44	—0·16	—0·74
4. 1·0 gm. Fe_2O_3	21·60	21·30	—0·30	—1·39
II				
Aluminium sulphate [$Al_2(SO_4)_3.18H_2O$] equivalent to—				
1. 0·25 gm. Al_2O_3	10·8	10·90	+0·10	+0·93
2. 0·5 gm. Al_2O_3	10·8	11·00	+0·20	+1·85
3. 0·75 gm. Al_2O_3	10·8	10·80	*Nil*	*Nil*
4. 1·0 gm. Al_2O_3	10·8	10·60	—0·20	—1·85
III				
Calcium carbonate ($CaCO_3$) equivalent to—				
1. 0·02 gm. CaO	10·8	10·70	—0·10	—0·93
2. 0·04 gm. CaO	10·8	10·60	—0·20	—1·85
3. 0·08 gm. CaO	10·8	10·60	—0·20	—1·85
4. 0·10 gm. CaO	10·8	10·90	+0·10	+0·93
IV				
Magnesium sulphate ($MgSO_4.7H_2O$) equivalent to—				
1. 0·02 gm. MgO	10·8	10·70	—0·10	—0·93
2. 0·04 gm. MgO	10·8	10·70	—0·10	—0·93
3. 0·08 gm. MgO	10·8	10·90	+0·10	+0·93
4. 1·00 gm. MgO	10·8	11·00	+0·20	+1·85

Experiment 5

This was conducted to estimate variable amounts of potassium oxide in the presence of different groups of bases when present in normal quantities as in soils. The experimental procedure adopted for the determination of potash in these complex mixtures is as follows :—

The mixture containing potash is evaporated to dryness on a water bath ; the residue is then extracted with hot water and filtered. About 5 to 10 c. c. of 10 per cent citric acid is then added to the filtrate depending on the amounts of iron and alumina present therein and the resultant mixture neutralised with sodium carbonate of similar strength. The neutralised solution is further rendered slightly acidic by the addition of strong acetic acid. Citric acid is added to avoid the precipitation of basic acetates, especially iron, by the free acetic acid in the reagent itself when the mixture is concentrated on a water bath. The potash solution is then evaporated to a pasty consistency, stirring repeatedly, especially towards the end, to break the crystalline mass adhering to the sides of the dish. After cooling, the precipitate is treated with 5 to 10 c. c. of 10 per cent acetic acid saturated with sodium cobaltinitrite precipitate of potassium washed with this solution and then filtered over a Gooch with an asbestos felt with a disc of Whatman No. 40 filter paper on it. For the preparation of the filter the asbestos was first digested with

potassium permanganate and subsequently with an excess of oxalic acid. The digested asbestos is further washed with distilled water till free from acid and then used. The potash in the precipitate is then determined volumetrically as in Drushel's [1907, 1908] method, outlined already in the beginning of the paper. From the values recorded in Table VI, it may be seen that there is practically no difference between the theoretical and estimated figures, considering the small quantities of potash handled. The percentage error works out to less than 2 in all cases.

TABLE VI

Effect of different groups of metallic radicals on the volumetric estimation of K_2O by the cobaltinitrite method

Mixture No.	Metallic salts used	K_2O added theoretical (mg.)	K_2O recovered, average of four determinations (mg.)	Deviation of the estimated value from the theoretical (mg.) + or —	Per cent error of K_2O recovered + or —
I	Ferrous sulphate equivalent to 0·3 gm. Fe_2O_3	5·40	5·43	+0·03	+0·56
		10·80	10·70	—0·10	—0·93
		21·60	22·00	+0·40	+1·85
		27·00	26·70	—0·30	—1·11
II	Ferrous sulphate equivalent to 0·3 gm. Fe_2O_3 + Aluminium sulphate equivalent 0·3 gm. Al_2O_3	5·40	5·48	+0·08	+1·48
		10·80	10·60	—0·20	—1·85
		21·60	21·20	—0·40	—1·85
		27·00	26·50	—0·50	—1·85
III	Ferrous sulphate equivalent to 0·3 gm. Fe_2O_3 + Aluminium sulphate equivalent to 0·3 gm. Al_2O_3 + Tricalcium phosphate equivalent to 0·02 gm. CaO	5·40	5·48	+0·02	+0·36
		10·80	10·70	—0·10	—0·93
		21·60	21·30	—0·30	—1·39
		27·00	26·80	—0·20	—0·74
IV	Ferrous sulphate equivalent to 0·3 gm. Fe_2O_3 + Aluminium sulphate equivalent to 0·3 gm. Al_2O_3 + Tricalcium phosphate equivalent to 0·02 gm. CaO + Magnesium sulphate equivalent to 0·02 gm. MgO	5·40	5·35	—0·05	—0·93
		10·80	10·80	*Nil*	*Nil*
		21·60	21·30	—0·30	—1·85
		27·00	26·70	—0·30	—1·11

Experiment 6

Application of the method to soils.—A convenient aliquot portion (50/500 c.c.) of the hydrochloric acid extract of about 20 grms. ignited soil, prepared in the usual manner is evaporated to dryness on a water bath and the residue extracted with hot water and filtered. The resulting solution is then treated with 5 to 10 c. c. of 10 per cent citric acid, depending on iron and other bases present and the whole rendered slightly alkaline with 10 per cent sodium carbonate. The excess carbonate is neutralised with acetic acid till the mixture is distinctly acidic. The potash is then precipitated and determined exactly on the same lines as for the artificial mixtures, using *N*/20 oxalic acid and permanganate for titration. For the experimental verification of this method, ten different soils representing important types from the various parts of the Presidency were taken, namely (1) black cotton soil, (2) garden soil, (3) paddy soil, (4) estate soil rich in organic matter, (5) orchard soil and (6) red soil. Potash is determined in hydrochloric acid

extracts of these soils both directly and after the removal of the bases by sodium carbonate. The results obtained by these procedures were compared with those obtained by the usual platinichloride method, Lindo Gladding modification. From Table VII it may be seen that the results from all these procedures agree with one another more or less very closely.

Table VII

Potash estimation by the cobaltinitrite (volumetric) and platinichloride methods in HCl extracts of soils

Types of soils used	K_2O by the cobaltinitrite volumetric procedure (average of two determinations)		K_2O by the platinichloride method—Lindo Gladding—average of two determinations (mg.)	Per cent recovery of K_2O by the cobaltinitrite procedure over platinichloride method	
	Without removing the bases (mg.)	After the removal of bases (mg.)		In presence of bases	In absence of bases
I. Black cotton soil—No. (1)	14·46	14·40	14·50	99·72	99·31
„ „ „ „ (2)	12·32	12·36	12·40	99·35	99·68
II. Garden soil—No. (1)	18·70	18·80	18·87	99·08	99·63
„ „ „ (2)	17·40	17·38	17·45	99·49	99·60
III. Paddy soil—No. (1)	14·08	14·18	14·10	99·84	100·56
„ „ „ (2)	12·18	12·10	12·12	100·49	99·83
IV. Red soil—No. (1)	7·60	7·58	7·62	99·74	99·47
„ „ (2)	10·06	10·12	10·10	99·60	100·20
V. Estate soil	24·12	24·30	24·20	99·68	100·40
VI. Orchard soil	28·10	28·18	28·21	99·61	99·89

Note.—Figures represent potash in 100/500 c. c. of the HCl extract of about 20 grms. of ignited soil for each experiment. Two different soils were used to represent each of the types from I to IV except in the case of V and VI.

The method recommended above for hydrochloric acid extracts of soils is a deviation from those adopted by many investigators in the field in certain respects and attempt has been made to simplify the procedure. The removal of the bases by sodium carbonate before precipitation recommended by Bowser [1909] has been found unnecessary, provided the citric acid procedure is resorted to. The introduction of citric acid in the medium does not in any way seem to interfere with the precipitation of potash and its chief function is to keep iron, alumina, phosphates, etc. in solution.

The aliquot taken for the determination of potash in the above experiments are 100/500 c. c. portions of the hydrochloric acid extracts of about 20 grms. ignited soil and these have been found very convenient to handle. To test the soundness of the method still further known quantities of potash in the form of potassium sulphate were added to 100 c. c. portions of the hydrochloric acid extracts of the typical soils and the total potash estimated volumetrically without removing the bases. A glance at Table VIII will show that practically almost all the added quantities have been recovered, thereby confirming the reliability of the method.

TABLE VIII

Recovery of added potash from HCl extracts of soils by the cobaltinitrite (volumetric) method

Soils used	K_2O in soil extract, chloroplatinate method (mg.)	K_2O added to soil extract (mg.)	Total K_2O in soil extract (mg.)	Total K_2O recovered by the cobaltinitrite method, average of two determinations (mg.)	Recovery of added K_2O by the cobaltinitrite method (mg.)	Per cent K_2O recovered over theoretical
Black cotton soil	14·50	5·40	19·90	19·88	5·38	99·63
Garden soil	18·87	5·40	24·27	24·24	5·37	99·44
Paddy soil	14·10	5·40	19·50	19·55	5·45	100·93
Estate soil	24·20	5·40	29·60	29·62	5·42	100·37
Red soil	10·10	5·40	15·50	15·51	5·41	100·20
Orchard soil	28·21	5·40	33·61	33·56	5·35	99·08

In the case of citric acid (1 per cent) extracts of soils, a convenient aliquot (containing 5 to 25 mg. of potash) from each extract is evaporated and ignited at a low temperature. The residue is dissolved in dilute hydrochloric acid, evaporated to dryness, gently ignited and extracted with 50 to 100 c. c. of boiling water. The filtered extract is rendered acidic with acetic acid and potash determined as in the case of pure salts. Known amounts of potash in the form of potassium sulphate were also added to citric acid extracts of soils before evaporation and ignition and the recovery of added potash effected almost completely. The results obtained are presented in Table IX.

TABLE IX

Recovery of potash from citric acid (1 per cent) extracts of soils by the cobaltinitrite (volumetric) method

Soils used	K_2O in soil extract, chloroplatinate method (mg.)	K_2O added to soil extract (mg.)	Total K_2O in soil extract (mg.)	Total K_2O recovered by the cobaltinitrite method, average of two determinations (mg.)	Recovery of added K_2O by the cobaltinitrite method (mg.)	Per cent K_2O recovered over theoretical
1. Black cotton soil	8·63	3·70	12·33	12·29	3·66	98·92
2. Garden soil	9·38	5·20	14·58	14·52	5·14	98·85
3. Paddy soil	5·76	7·50	13·26	13·18	7·42	98·94
4. Estate soil	10·42	5·40	15·82	15·75	5·33	98·70
5. Orchard soil	5·84	8·10	13·94	13·89	8·05	99·38
6. Red soil	4·32	10·80	15·12	15·08	10·76	99·63

NOTE.—The recovery of potash is almost quantitative in each case.

Summary

1. The cobaltinitrite method of estimating potash, both volumetrically and gravimetrically, has been tested with reference to simple salts, artificial mixtures of various bases, and acids, as well as hydrochloric acid and citric acid extracts of soils. It has been found reliable and quick within the ranges tried—3 to 53 mg. of potassium oxide. The volumetric procedure especially may be of immense use in determining small quantities of potash, even below 1 mg. as are usually met with in citric acid and ammonium chloride extracts of soils.

2. The method is least expensive and can be applied in the presence of sulphates, phosphates and other bases and in these respects it has distinct advantages over the platinichloride and perchlorate methods.

3. A simple modified volumetric procedure of the method for the direct estimation of potash without removing the bases in the hydrochloric acid extracts of soils has been outlined.

Acknowledgments

The author is greatly indebted to Rao Bahadur B. Viswanath, F.I.C., F.C.S., Director, Imperial Agricultural Research Institute, New Delhi (formerly Agricultural Chemist to the Government of Madras), for his kind help and encouragement in the conduct of this work.

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ABSTRACTS

A New Species of Azotobacter. Robert L. Starkey and P. K. De (*Soil Sci.*, Vol. 47, No. 4, 1938)

During the course of an investigation of nitrogen fixation in rice soils of of India several cultures of nitrogen-fixing bacteria were isolated. From two acid soils cultures were obtained which were alike among themselves but different from any previously described species of *Azotobacter*. In consideration of these differences the organism was described as a new species, and named *Azotobacter indicum*.

The two soils were more acid than those from which *Azotobacter* is commonly recovered : Dacca soil of *p*H 4·9 and soil from Insein, Burma of *p*H 5·2. The organism was isolated on silica gel plates containing sucrose. Pure cultures grew well in nitrogen-free media containing sucrose and glucose. Dextrin was not utilised. Although development was very slow upon mannite, considerable amounts of nitrogen were fixed in a solution medium containing this material during prolonged incubation. In the presence of $CaCO_3$ growth was very much depressed.

In comparison with other species of *Azotobacter* the new organism grew very slowly ; visible growth could seldom be detected before three to four days at the incubation temperature of 30°C. The surface colonies were uniformly turbid and starchy in appearance, and were very much raised, round and dome-shaped. The colonies fused into mucoid masses when they was not well separated from one another. Development on solid media was characterised by the production of a profusion of slime which gave the cell material a very mucoid, gummy and tenacious consistency. It could be drawn out in long threads with a needle and was somewhat elastic. The material was at first colourless but became buff to reddish brown during incubation for three weeks or longer.

The organism failed to grow on nutrient agar, or on peptone agar but developed slowly upon a medium containing both peptone and glucose and in other media containing carbohydrate in addition to nitrogenous organic materials.

The cells were Gram-negative and motile and measured 0·5—1·2 × 1·7—2·7μ. They differed principally from all other species of *Azotobacter* in that there were large fat globules in the cells. Generally there was one large globule at each end of the rod-shaped cells, but cells were also seen with only one terminal globule. A large portion of the cell, frequently more than one-half of the total volume, was occupied by the fatty material. The cells changed very slightly in morphology during prolonged incubation in

nitrogen-free carbohydrate media but became abnormal in media containing certain nitrogenous materials. In the latter case some of the cells were much larger, whereas others were swollen, elongated, unsymmetrical, budded, or yeastlike. No cysts or spores were detected under any condition.

The amounts of nitrogen fixed by *Az. indicum* were comparable to those fixed by other species of *Azotobacter*. On silica gel plates, between 7·0 and 9·8 mg. were fixed per plate containing 1·0 grm. of sucrose. In later tests there was an average of 6·0 mg. per plate with 1·5 grm. of glucose. Between 3·1 and 9·0 mg. of nitrogen were fixed per flask of solution medium containing 1·5 grm. of glucose or sucrose where $CaCO_3$ was added. In later experiments without $CaCO_3$ there was much greater fixation; between 13·5 and 19·4 mg. of nitrogen were fixed in solution media with glucose or sucrose and 9·9 mg. with mannitol.

Development in solution media was characterised by strong turbidity, by the accumulation of considerable sediment which increases as the incubation period was prolonged, and by the formation of a ring of slimy material on the wall of the flask at the surface of the liquid. There was no pellicle and practically no evidence of pigmentation. (*Authors' abstract*)

Sodium Ferrocyanide as Dispersing Agent in Mechanical Analysis of Soils.

Baradananda Chatterjee and Basanta Kumar Das

(*Soil Research*, Vol. **6**, No. 4/5, page 227, 1939)

The pre-treatment of soil with acid and the final dispersion in a highly alkaline medium in the mechanical analysis of soils are open to criticism. A procedure has been adopted in which these two undesirable treatments are omitted. The soil is treated with 6 per cent H_2O_2 for removing the organic matter. It is then leached with about 500 c.c. of normal NaCl, then twice with 100 c.c. 0·01*N*—NaCl and finally with distilled water. The final dispersion is made in 0·002*M* $Na_4Fe(CN)_6$ by shaking and the suspension is subjected to pipette sampling. It has been found that—

(*i*) dispersion by $Na_4Fe(CN)_6$ is as efficient as in the International-soda method,

(*ii*) final dispersion is affected at much lower *p*H; and

(*iii*) even laterite soils can be efficiently dispersed by $Na_4Fe(CN)_6$ at a *p*H as low as 6·47.

The addition of increasing amounts of NaOH to ferrocyanide, though increases the *p*H of the suspension, does not materially increase it dispersing action on the clay. The efficient dispersion of soils by $Na_4Fe(CN)_6$ has been ascribed to the adsorption of polyvalent anions by the negatively charged oil colloids. (*Authors' abstract*)

NOTE

NOTICE NO. 2 OF 1939

PLANT Quarantine Regulations and Import Restrictions relating to the following countries have been received. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research.

United States Department of Agriculture, Bureau of Entomology and Plant Quarantine—

1. White fringed beetle quarantine (No. 72)

2. Information for the press—Revised pink boll-worm quarantine and restrictions

3. Service and regulatory announcements, October-December 1938

Summaries of the Plant Quarantine and Import Restrictions, issued by the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine of :—

4. Albania
5. British Gold Coast Colony
6. British Colony of Ceylon
7. Kingdom of Italy
8. Commonwealth of Australia (Supplement No. 2)
9. Chosen (Korea)
10. Kingdom of Yugoslavia (Supplement No. 4)
11. Iran

Notice regarding the Regulations for the prevention of disease amongst plants.

REVIEWS

The Rape of the Earth : A World Survey of Soil Erosion. By G. V. Jacks and R. O. Whyte. Faber and Faber. 21*s*.

Judging from the large amount of concrete evidence produced in many other countries of the drier tropics and semi-tropics, soil erosion must be accepted as a serious fact and not a mere subject for dialectics and debate. It is not confined to the United States of America nor is it another American newspaper man's publicity stunt, although American papers continue to give this subject good front page value as news. It is proved by this book, if proof were needed, that almost every country of the world is suffering to some extent from preventable soil losses and that India is undoubtedly one of the worst sufferers. The authors who are both recognised authorities each in his own field, Jacks as a soil chemist and Whyte as a grassland ecologist, consider that a national catastrophe due to soil erosion and its dreadful consequences is perhaps more imminent in South Africa than in any other country. General Smuts, quoted in this book, has stated that " erosion is the biggest problem confronting the country, bigger than any politics ". In spite of the publicity focussed upon this subject in America the authors consider that the African continent must now take the foreground for erosion " news value ". In the United States of America the federal and states governments are teaching the farmers to grapple with local phases of the erosion problem and this work is now going forward on an immense scale and in an orderly and organised fashion, whereas in Africa the need for soil conservation measures is even more urgent but the problem cuts across the social customs of diverse races, the habits of life of ignorant and suspicious pastoral tribes, conflicting interests of innumerable forms of government, and demands of white and black populations, sometimes mixed, sometimes segregated.

But where does India stand between these two extremes and how can Indian land-use practices be improved in the light of this newly acquired knowledge in these other continents ? This is what the authors say of India : " Over much of India the people have accepted a slowly deteriorating environment as part of the scheme of things ; the vast majority is probably unaware that erosion is occurring." This is unfortunately a painfully true statement, and makes the introduction of control measures a much more difficult task than in countries where every farmer is able to understand the danger and willing to take his share in erosion control measures.

Of the two great causes of erosion damage, grazing is given considerably more space than field cultivation for the world as a whole and for India in particular, but this does not necessarily reflect the importance of their relative contributions. The actual steps necessary to obtain control of soil erosion are already known, but it is quite another matter to put them into effect against

the opposition of conservative and suspicious villagers. The social repercussions of attempts at control of grazing are usually much more serious than in the more straightforward issues of field crops and plough-lands, and as due weight is given to this very important side of the work, the control of grazing on wild lands is dealt with in considerable detail. The authors agree that constructive work can best be done on a basis of self-help and free labour contributions, because provincial governments can hardly be expected to undertake this for each of the thousands of villages which require attention. The two main heads for village activity to be dealt with, preferably through their own Co-operative Society or Panchayat, are : (1) to develop fodder resources for local live-stock, and (2) to improve wild lands so as to conserve moisture and reduce run-off. Common and useful lines of attack are :—the replacement of grazing by grass-cutting ; rotational closure of grazing land ; partition of *shamlat* or common waste land ; intensive improvement of natural grasslands by means of " gully plugging " with small checkdams of any material which may be locally available ; contour ridging and trenching to encourage better grass growth on ground closed to grazing ; determination of the correct grazing incidence for local types of bush and grass ; development of tree fodder supply with controlled rotational lopping ; encouragement of green fodder crops and silage ; and most important of all, the reduction of herds by a campaign for fewer and better cattle.

To most of our readers, however, the main interest is probably in control measures as applied to field crops, and it is interesting to read these experts' opinions. Great emphasis is laid on the need for imitating the American drive for soil conservation measures in plough-land, but they point out the dangers of slavish copying of unsuitable or untried methods. Much can be suggested from an empirical knowledge of control measures used elsewhere, but the great need in India at the moment is to work out a local technique for the many variations in crops and types of soil and slope, particularly as affecting local recommendations for contour ridging (*watt-bandi*) practice. The local method of bund-building varies from place to place according to the caste of the cultivator. For instance a Jat village can be trusted to have a large percentage of its fields properly *watted* while neighbouring Rajputs and Gujars may not even have started. When the use of the ox-drawn wooden scoop or *karah* is universal in the rest of the Punjab why is it almost entirely unknown in a comparatively central area such as Gurdaspur ?

At the moment there is considerable enthusiasm amongst civil officers for advocating *watt-bandi* without sufficient knowledge of the serious effect upon certain crops through the building of large field *watts* or bunds. Cotton, maize and *bajra*, for instance, are liable to suffer badly from sun-scorch if they stand for two or three days in water which has been caught behind a two-feet high bund. The reviewer recommends as a safer alternative for such crops to multiply the number of parallel bunds and make them much smaller and closer together, say six inches high and eight to ten feet apart, so that the water caught behind each will have seeped away in a few hours. In the case of light *barani* soils used for winter wheat and left as a bare fallow in summer, there is

no danger whatever in building larger *bunds* further apart ; the urgent point here is to get the fallow ploughed before the monsoon breaks and to keep it broken by two or three subsequent monsoon ploughings to ensure good seepage. The book under review issues repeated warnings on the subject of bare fallow, but so far India has not found any panacea for the sheet erosion from fallow fields like the *Lespedeza* now used in some variety or other for sowing on fallow in a great many parts of the United States of America. Here lies a brilliant opportunity for the Provincial Agriculture Departments to work out their own and the villagers' salvation by finding a cheap and handy leguminous or grass plant which could be sown on fallow to protect the soil from the onslaught of monsoon storms ; it should also produce some fodder and improve the soil when ploughed in as a green manure.

It is interesting to read that in almost every country it is the method of land tenure that forms the worst stumbling-block to the introduction of control in plough-lands. Tenant farmers never seem to get the best out of the land, because it tends to deteriorate when it is worked without considering the long-term consequences. Short leases prevent the use of soil-improving crops or of a rotation of crops. It would appear therefore that improvement lies in the hands of the Land Revenue staff because the *patwari*, guided by the revenue assistant, can bring some weight to bear on such problems. The existing revenue staff is, however, fully occupied with its routine work and can hardly be expected to undertake extra tasks. Another possible alternative is to enlarge the scope of the special co-operative staff which now handles the consolidation of holdings, and make it responsible for the partition of *shamlat* and the encouragement of *watt-bandi*. In many of the foothill villages consolidation is of practical importance only in the intensively cultivated home-fields nearest to the dwellings, *watt-bandi* is badly needed in all the rest of the out-field land, while partition may be desirable in the *shamlat* to encourage the creation of hay-fields or the reclamation of gullied waste.

A part of the authors' soil conservation recommendations which has been sadly neglected so far in India is tree shelter-belts. A great deal of shelter-belt planting was undertaken in Russia in the more arid parts of the wheat-growing steppe some 60 years ago and the results have been so satisfactory that the Soviet is now carrying out a programme for establishing nearly 900,000 acres of shelter-belt in their current Five Years' Plan. The United States of America is also forging ahead with its $1\frac{1}{4}$ million acres of planting in the Great Plains shelter-belt project. In the Californian citrus-growing districts which are similar in many respects to the irrigated fruit farms of the Punjab, shelter-belts are considered absolutely essential ; 10 per cent of the area of each Californian garden is usually kept under wind-break trees and as much as 40 rupees per orchard acre is budgetted for wind-break maintenance. With the extremes of heat, drought and cold which the Punjab experiences it is surprising that so little attention has been paid to this question, and throughout India there is a great need for the closer union and co-ordination of forestry with agriculture in dealing with the everyday problems of the cultivator. This book should be in the hands of everyone who is in any way responsible for the growing of plants and the produce of the soil. [R. M. G.]

Fruit Juices and Related Products. (*Technical Communication No. 11 of the Imperial Bureau of Horticulture and Plantation Crops, East Malling, Kent, England*). By V. L. S. Charley, and T. H. J. Harrison, 1939. Figures 49. Bibliography 118. Price 5*s*.

Whether we have suddenly developed sense, or whether the old slogan "an apple a day keeps the doctor away" has at last struck home, is immaterial. The fact remains that we do eat more fruit and that more fruit is being produced. Market quality standards have become higher and growers are now faced with the problem of how to dispose satisfactorily of fruit which does not come up to the accepted standards of size or colour, though otherwise perfectly good. Among processes offering a solution, that of unfermented juice production has long commended itself to fruit producers in Europe and the U. S. A.

Hitherto, though short articles on different aspects of processing have been available since 1914 in the annual reports of the Long Ashton Research Station, lack of precise information on the exact technique employed has checked progress in England.

It has remained for Charley to give under one cover a complete detailed account of the manufacture of apple juice based on his knowledge of the latest foreign methods and on his own continued investigations at Long Ashton.

In the present bulletin he deals with the manufacturing process in detail, starting with the selection of the fruit and taking us through every stage in turn up to the disposal of the final product in bottle, can or other type of dispenser. He does not confine his remarks to any one process but gives particulars of different methods found satisfactory and discusses their merits.

Further he deals more briefly with such other fruit products as grape, citrus and soft fruit juices, cider, fruit wines, etc., with the concentration of fruit juices by hot processing, by freezing and by spray drying, and with the disposal of pomace.

He describes methods of analysing juice for the determination of specific gravity, total acidity, tannin, alcohol and sulphur dioxide.

Finally he discusses the suitability of particular metals and alloys for the construction of the requisite apparatus, the clear illustrations of which add greatly to the usefulness of the publication.

But the problems of juice production do not, unfortunately, begin and end with its manufacture, and the economic and nutritional aspects are equally important. We are, therefore, grateful to Harrison for his concise and adequate treatment of them in the first twenty pages.

He shows that a permanent fruit juice industry on a large scale can only be developed where large supplies of raw material are available each year at a reasonable price. The cost of manufacture is not small, and if sales are to grow it is essential that retail prices should be reasonably low so as to compete with other drinks. This means that distribution costs must be low and that the industry must depend essentially on its home market. Here regulations are necessary to protect it from competition with synthetic products.

The nutritive and therapeutic qualities of fruit juices are discussed at sufficient length to show their great value, not only to children and invalids and for the treatment of gastric troubles, but also in the diet of the normal healthy adult.

A short glossary is included for the use of the less expert, and this together with the comprehensive index should make the information readily accessible to all.

The Action and Use of Colchicine in the Production of Polyploid Plants. By J. L. Fyfe. Issued by the Imperial Bureau of Plant Breeding and Genetics (for Crops other than Herbage), Cambridge. Pp. 10. Price 1*s*.

The discovery of the action of the drug colchicine on nuclear division, making it the most reliable agent yet used in the production of polyploid plants, has excited great interest even in laymen and is of importance to those working on the cytology or genetics of plants.

The Imperial Bureau of Plant Breeding and Genetics, Cambridge, has issued a bulletin on the subject, summarising the already fairly extensive literature. This bulletin begins by explaining the chromosome doubling; next a detailed account of the action of colchicine on mitosis and meiosis is given, followed by summaries of the results obtained from the use of colchicine for producing polyploid plants. The action and use of acenaphthene, which the Russian workers have shown to be a similar agent to colchicine are also described. Particular attention has been paid throughout to treatments and dosages and some recommendations are given which should be helpful to those contemplating the production of polyploids. All the literature appearing up to the end of January 1939 is included and listed in a bibliography of 38 references.

Bibliography of Baking Quality Tests (Supplement). Issued by the Imperial Bureau of Plant Breeding and Genetics (for Crops other than Herbage), Cambridge. Pp. 10. Price 1*s*. 6*d*.

The valuable "Bibliography of Baking Quality Tests" issued by the Imperial Bureau of Plant Breeding and Genetics in 1934 has been brought up to date by the publication of a supplement which contains titles of papers from 1933 to 1938 inclusive.

Some of the papers noted have been abstracted in " Plant Breeding Abstracts " but many, because of their specialist nature, have not been reviewed but will thus become available to workers on the subject.

A note of volume and abstract number in the margin indicates where an abstract may be found in " Plant Breeding Abstracts ". The titles are arranged alphabetically according to authors and, given sufficient demand, further supplement are promised from time to time.

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